NURSERY REARING OF PEARL OYSTER *PINCTADA FUCATA* (GOULD, 1850) UNDER ONSHORE CONDITIONS

Thesis submitted in partial fulfillment of the requirements for the degree of

Ph.D. in Fish & Fisheries Science (Mariculture)

by

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MAY 2002

To
My beloved Parents and Sister

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CERTIFICATE

Certified that the thesis entitled **"NURSERY REARING OF PEARL OYSTER *Pinctada fucata* (GOULD) UNDER ONSHORE CONDITIONS"** is a record of independent bonafide research work carried out by **Mr. Ansy Mathew, N. P.** during the period of study from September 1998 to May 2002 under our supervision and guidance for the degree of **Doctor of Philosophy in Fish & Fisheries Science (Mariculture)** and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled "**NURSERY REARING OF PEARL OYSTER *Pinctada fucata* (GOULD, 1850) UNDER ONSHORE CONDITIONS**" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

पूर्ण अवस्था में अनुकूलतक सांढ़ में सीपिया *पिनटाडा फूकाटा* जलांडक का सफलतापूर्ण पालन - पोषण और वाणिज्यपरक उत्पादन में सीपि के बीज के उच्च जीवित रहने का दर और विकास के लिए उचित चारा का उपयोग करना महत्वपर्ण है । इन पहलूओं के महत्व को ध्यान में रखते हुए यह अध्ययन केंद्रीय समुद्री अनुसंधान संस्थान विशाखापटणम के क्षेत्रीय कार्यालय में किया जा रहा है । अनूकूलतक स्टोकिंग सांढ़, माइक्रो अलगेल आहार की गुण और मात्रा और डायटेरी माइक्रोलेज का रासायनिक सम्मिश्रण आदि तटीय सीपी संवर्धन के विशिष्ट पहलूओं का अध्ययन है। इस अध्ययन का परिणामों का महत्वपूर्ण है । . उच्च सांद्रों में भी १०० % जीवित दर का किस्साई देता है । आइसोक्रिसिस गलबाना और स्केलीटोनिया कोसटाटम को लोड दें तो , ६० कोशिका से गाढापन एक ही जाति कि आहार में सीपी का अधिकतम वृद्धि हुई । इस अध्ययन से आलगल कोनसेंट्रेशन की वृद्धि में कम विकास दर यह दिखाता है कि ६० कोशिका के उपर सीपी आहार कोनसेंट्रेशन के एफिशिऐंट फीडिंग अनूकूल नहीं है ।दो डायटिम जाति, एसोक्रिसिस गलबाना और केटोसीरोस कालसिट्रान्स ज्यादा विकास दर दिखाया । यही अन्य तीन जातियों के लिए अच्छा है या फिर अच्छा विकास दर दिखाए पाँच आलगेल जाति का कोंबीनेशन अच्छा रहेगा । यह अध्ययन यह दिखाता है कि पी फूकाटा में फीडिंग और अबसोपशेन आहार की उपलब्धता से संबद्ध है । आलगल जाति का रिलेटिव आहार मूल्य क्रम में था । आई गलबाना, सी कालसिट्रान्स , एस कोस्टाटम , एन सलिना ,टी ग्रासिलस ।माइक्रो आलगे की पाँच जाति का प्रोटीन स्तर २३ - २९ % सूखा वजन के रेंज में फर्क दिखाता है । आई गलबाना ,सी कालसिट्रान्स ,एस कोस्टाटम,एन सलिना , टी ग्रासिलस का लिपिड लेवल (१९%, १७%, १२%, ८% और ६% क्रमशः सूखा वजन है ।प्रस्तुत अध्ययन में आई गलबाना ,सी कालसिट्रान्स ,एस कोस्टाटम,एन सलिना , टी ग्रासिलस का कारबोहैड्रेट लेयल ७%, ४% , ८%, ६%, और ७% क्रमशः सूखा वजन है ।

ABSTRACT

Successful rearing of spat of the pearl oyster, *Pinctada fucata* (Gould, 1850) in ideal conditions in optimum density and using appropriate feed is of utmost value for high survival rate and growth of pearl oyster seed in commercial production. Considering the importance of these aspects, the present study has been undertaken at the Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute to understand the various nursery rearing of pearl oyster under onshore conditions such as stocking density, quality and quantity of microalgae condition, nutritional quality indicated by biochemical composition. A significant aspect of the results in the present study is the 100% survival rate observed even in higher densities. The concentration of 60 cells/µl resulted in maximum growth of pearl oyster spat in single species diets, except for *Isochrysis galbana* and *Skeletonema costatum*. The decreasing growth rates observed in the present study with increase in algal concentration suggests that the pearl oyster is not adapted for efficient feeding at higher food concentrations above 60 cells/µl. The two dietary species, *Isochrysis galbana* and *Chaetoceros calcitrans* showed a better growth rate. The same also holds good for other combinations of three species diets or a combination of all the five algal species, which showed remarkable growth. The present study has shown that feeding and absorption in *P. fucata* are significantly correlated with food availability. The relative food value of the algal species examined were in the order *I. galbana*> *C. calcitrans*> *S. costatum*> *N. salina*> *T. gracilis*. The protein levels of the five species of microalgae have been observed to vary over a limited range of 23-29% dry weight. The lipid level of *C. calcitrans*, *I. galbana, S. costatum, N. salina* and *T. gracilis* are in decreasing order of 19%, 17%, 12%, 8% and 6% dry weight respectively. The carbohydrate level of *C. calcitrans*, *I. galbana, S. costatum, N. salina* and *T. gracilis* in the present study are low 5%, 4%, 8%, 6% and 7% dry weight respectively.

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1. INTRODUCTION

Tockichi Nishikawa succeeded in producing the first spherical cultured pearl in the pearl oyster in Japan in 1907 by introducing a shell bead nucleus and a piece of mantle into the body of a pearl oyster, the latter secreting pearly nacre around the bead. Following this landmark breakthrough, pearl culture industry came into existence in Japan and cultured pearls have come to be produced in increasing quantities in pearl farms set up in sheltered coastal waters of the country. After Second World War pearl culture industry has been established with the Japanese collaboration in Australia, Philippines, Burma, Thailand, Indonesia and Malaysia. In India, experimental work on pearl culture was carried out in Tamil Nadu, since 1938 and in Gujarat since 1958. Success has been achieved in developing the pearl culture technology and in 1973 the first batch of free, spherical cultured pearls has been produced in the Indian pearl oyster *Pinctada fucata* (Gould, 1850) by the Central Marine Fisheries Research Institute (CMFRI) at Tuticorin.

Pearl fisheries of India in the Gulf of Mannar and Gulf of Kutch are well known for yielding one of the finest natural pearls. The fishery was based on the Indian pearl oyster, *Pinctada fucata*. Chacko (1970) and Mahadevan and Nayar (1973) have described the pearl fisheries of this region. From the account of the *paars* fished, number of oysters landed and the effort spent, it is evident that the oysters ranging in length from 55-75 mm in the age group of 3 – 3 ½ years formed the fishery. The availability of oysters in natural beds vary widely and the production of pearls have declined over the years. Mahadevan and Nayar (1968) reported that the pearl oysters were very few after the post fishery season as most of them having been fished and the remaining either perished or eaten away by predators. Another species of importance for culture is the black-lip pearl oyster, *P. margaritifera* found in the Andaman and Nicobar Islands. Although other species such as *P. chimnitzii*, *P. sugillata* and *P. anomoides*

occur in the Indian waters, they are not useful in the production of cultured pearls. The lack of adequate natural resources of the perl oyster *Pinctada fucata* for commercial pearl culture has been solved by the development of hatchery technology for spat production of *P. fucata* by CMFRI in 1983. It has been demonstrated that over a million spat can be produced from one set of larval rearing in the hatchery (Alagarswami *et al.*, 1987a).

Pearl oyster rearing is the major component of pearl culture. This is a continuous operation with rafts, long lines or bottom platforms maintained in position in the sea round the year. The various methods of rearing oysters in the sea are the raft culture, collapsible rafts and on bottom culture. Selection of site is an important factor of the farming and a sheltered bay with protection from the wind and the wave action offers an ideal site for farming the pearl oysters. Farming husbandry includes the maintenance of optimum stock size, increasing survival rate, farm maintenance, monitoring of growth and reproduction and control of boring and fouling organisms. The environmental parameters that need to be considered are primary productivity, temperature, salinity, depth, nature of bottom, water current, proximity to river flow and silt load. The hydrographic conditions in the sea fluctuate widely with changes in season. Rough sea conditions during monsoon season and cyclones also hamper the farming operations. The occurrence of fouling and boring organisms is another major factor that affects farming at sea (James, 1987).

The early studies on the growth of pearl oysters in tanks was attended by Kuwatani and Nishi (1969), Kuwatani *et al.* (1969), and Kuwatani *et al.* (1974). They observed the combination of factors such as pH, alkalinity ammonia, nitrite, nitrate controls the growth of pearl oyster when cultured in tanks. They also reported that gonad development of pearl oyster for artificial spawning might be induced by the increased temperature especially in winter.

The Central Marine Fisheries Research Institute (CMFRI) has attempted the development of a technology for culturing pearls in onshore tanks. Growth of

pearl oysters in the cement tank with phytoplankton feeding was found to be high. Maximum growth rate was obtained at 70,000 cells/ml to 75,000 cells/ml of *Chaetoceros* (Rao and Devaraj 1996). The growth rates observed in this study are the fastest (8.0 mm to 26.75 mm in 56 days) observed in India making it possible to obtain adult oysters for implantation in about 6 months under on-shore tank conditions. The results have shown that onshore pearl culture is less risky and more economical when compared to open sea pearl culture. It provides an opportunity to maintain all key environmental factors together at the optimum level through good planning and management to make pearl culture successful.

The hatchery production of pearl oyster spat ensures mass production of pearl oyster spat, which can be utilized for pearl culture. It also ensures the availability of quality seed with uniform growth rates and higher survival rates.

The efficiency of a nursery system is determined by the stocking densities used. Growth rate of individual oyster spat decreasing with increase in stocking density. It has been clearly established that apart from temperature, salinity and food requirements, optimum stocking density is an important requirement for high growth rate. Proper stocking density results in maximum growth and minimum mortality in a culture system. Density of oysters in culture practices (20/60l) compares well to the density observed in natural water system where there is optimum growth. Stocking density also determines the rate of consumption of food, oxygen and dissolved organic compounds by the animals. It also determines the rate of production of wastes and the tolerance of animals to various water quality conditions. Stocking densities vary according to the stage of growth and from species to species. Although low densities result in good growth and low mortality, but will affect economic viability.

Several studies have been conducted to assess the value of algal species as food of oysters and oyster larvae (Bruce *et al.*, 1940; Loosanoff and Davis, 1963; Walne, 1964; Alagarswami *et al.*, 1983, 1989). The two algal species, *Pavlova lutheri* and *Isochrysis galbana* are widely used in pearl oyster hatcheries

in India and other countries. Commercially important oysters are cultured in natural conditions and grow on diverse natural diets, which vary depending on location, season, and environmental conditions. In hatcheries, however, algal diets have been used since the 1940s.

The first step in any feeding programme is the estimation of nutrient requirements. Very little is known about the essential and optimum aminoacid requirements in food protein and vitamin requirements of oysters. The importance of the 20:5ω3 and 22:6ω3 fatty acids for oysters is now well recognized and hatcheries are now using combinations of algal species that meet the fatty acid requirements of oysters. Algal diets, either singly or in combination have been found to be excellent food for oyster larvae and spat (Bruce *et.al.*, 1940). Algae concentrated into pastes or slurries have also been used to feed oyster larvae in laboratories and in commercial hatcheries. But these methods have yielded only partial success due to the denaturation of algal cells and acceptability by larvae (Nell, 1993). As hatchery production of spat is usually dependent on the expensive production of microalgae, efforts have been made to develop artificial dietary supplements as substitutes. These include the use of microparticulate diets, lipid-walled microcapsules and non-encapsulated diets. Marine yeast and bacteria have been tried as diets for scallops, mussels, clams and oysters. The major attraction is that yeasts and bacteria can be produced much more rapidly, efficiently and economically. They also have shorter generation time, higher cell densities and need inexpensive growth substrates. The yeast *Candida utilis* has been used successfully to replace up to 50% of the algae in the diet of the American oyster *Crassostrea virginica* spat (Epifanio, 1979).

The feeding mechanism in oysters depends on the ciliary action of gills driving a current of water through the ostia. During passage through the gills, particulate matter is filtered out, trapped in mucus, and transported to the labial palps, with which it is ingested in the mouth. Selection of food, therefore,

depends on particle size. Both filtration efficiency and ingestion are also determined by particle size. Food concentration or the amount of the food suspended in seawater, is an important criterion to be considered in the feeding of pearl oysters. Feeding rate, which is the daily amount of food supplied per oyster, is also an important factor for rearing larvae in hatcheries and spat in nurseries.

The pearl oyster, *Pinctada fucata* (Gould) was bred in India during the early 1980s by Central Marine Fisheries Research Institute at Tuticorin and an experimental hatchery has been established for mass production of spat. The feeding of larvae is with pure cultures of the two flagellates *I. galbana* and *P. lutheri*. The spat were fed a combination of algal diet containing *Chaetoceros calcitrans*, which enhanced the juvenile growth. Spat are then transplanted to farm in net cages for further growth in natural conditions (Chellam *et. al.*, 1987). The present study aims at understanding the algal requirements of the spat of *P. fucata* in onshore culture system. Investigations were also made on the effect of different food concentrations on the feeding and also on the relative importance of various species of algae either singly or in combination.

The nutritional quality of the food given to cultured marine organisms is crucial in the larval and juvenile life of bivalve mollusks (Nell, 1992). Marine microalgae are used extensively in mariculture as food particularly for larval and juvenile molluscs, crustaceans and fish. The proximate chemical composition of algae is generally regarded as species specific and is usually regulated by environmental factors. Biochemical content of cultured algae may also vary with age of culture and the nature of the nutrient culture medium. In general it is observed that no single species can fulfill the entire nutritional requirements of a particular species of bivalves (Nell, 1992). Factors such as the size, toxicity, digestibility and biochemical composition of the microalgae account for the differences in their food values.

With regard to biochemical composition, the total level of protein, lipid and carbohydrate may vary substantially with species and culture conditions. A precise knowledge of the composition could make it possible to determine algal diets to meet the requirements of different animal species. Recent studies have highlighted the importance of polyunsaturated fatty acids (PUFAs) as essential requirement of marine animals (Wikfors *et al.*, 1996, Volkman *et al.*, 1993). Microalgae lacking PUFAs have proved to be inadequate as unialgal diets, whereas species with high concentrations of the PUFAs have generally been proved to be good food for molluscan juveniles (Wikfors *et al.*, 1996, Volkman *et al.*, 1993). Polysaccharide levels of algae are also of nutritional significance as the efficiency with which marine animals digest polysaccharide depends on the polysaccharide type. Inspite of a large amount of information on the biochemical composition of algae, the specific nutrient requirements of molluscan larvae and spat are not completely known. Some authors point out the role of carbohydrates and lipids (including PUFAs) in algae in the growth of oysters (Wikfors *et al.*, 1996, Volkman *et al.*, 1993), whilst others (Sukeniki and Wahnon, 1991; Tatsuzawa and Takizawa, 1995) consider that the importance of food given depends much on other nutrients, fatty acids, amino acids, monosaccharides, minerals and vitamins, than on the gross biochemical composition.

Many studies have been conducted to understand the influence of diet on the chemical composition of marine animals. The biochemical composition of oysters also varies during the growth phase depending on season (Holland and Hannant, 1974). The chemical composition of oyster spat generally reflects that of dietary microalgae. The nutrient status of the cultures can also have a marked effect on the chemical composition of the algae, which in turn can greatly affect the growth of oysters. Carbohydrates are the main energy source for both juveniles and adult pearl oysters. The requirements of protein, lipid and carbohydrate vary with size, growth rate and the method of rearing. Dietary constituents have to provide not only the energy required for growth and

sustenance, but also those compounds which are not synthesized by the animal (Nell, 1993).

Successful rearing of spat of the pearl oyster, *Pinctada fucata* in ideal conditions in optimum density and using appropriate feed is the key for high survival rate and growth of pearl oyster seed in commercial production.

Considering the importance of these aspects, the present study has been undertaken at the Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute (CMFRI) to understand the various aspects of nursery rearing of pearl oyster under onshore conditions such as optimal stocking density, quality and quantity of microalgal food and biochemistry of food and tissue of pearl oyster spat. This work has been carried out with the following objectives:

1. To understand the optimum stocking density of spat under on shore tank conditions.
2. To evaluate the various microalgae either singly or in combination as a source of food for pearl oyster spat, and
3. To estimate the biochemical composition of algae and spat.

2. REVIEW OF LITERATURE

2. 1. SPAT DENSITY

Traditional type of pearl culture relies on the collection of pearl oysters from the wild. Adult oysters or spat set on suitable spat collectors are grown to the optimum size for use in cultured pearl production. Large scale pearl culture by this method may not be possible due to limited natural stocks of oysters and spat. Hatchery production of spat is the only viable alternative for obtaining pearl oyster seed. Therefore, the development of hatchery techniques for pearl oysters has been rapid in the recent past (Alagarswami, *et al.*, 1987a; Alagarswami *et al.*, 1989; Rose and Baker, 1994; Southgate and Beer, 1997).

Pearl oyster farming is the main component of pearl culture. It involves rearing of pearl oyster spat, in cages tied to rafts, long lines or bottom platforms in position in the sea round the year, removing filamentous algae and encrusting organisms like polychaetes and barnacles which adhere to the pearl oysters. The hydrographic conditions in the sea fluctuate widely with changes in season. Rough sea conditions during monsoon and cyclones also hamper the farming operations. The occurrence of fouling and boring organisms is a major factor that affects farming at sea.

An important component of any mariculture operation is the determination of optimum stocking density. This will ensure the seed survival and have suitable environmental conditions for maximum growth. An inverse relationship exists between concentration of spat and rate of growth (Loosanoff and Davis, 1963). Slower growth may be attributed to be deterious effect of greater concentration of excretory products and the availability of less food per spat. Crowding is undesirable as it decreases rate of growth and overcrowded spat are more susceptible to diseases than young ones in less densely populated cultures.

Chellam *et al.* (1987) have recommended different stocking densities in relation to size of spat in box cages suspended from rafts in sea. Stocking density of clam seed for bottom culture varies in different countries (Narasimham and Laxmilatha, 1996). Rose and Baker (1994) have stocked spat of *Pinctada maxima* at densities ranging from 4-25 spat /100 cm^2 and Southgate and Beer, (1997) observed the growth of *P. margaritifera* spat in plastic trays and pearl nets at different densities over a 19-week growth period. Rose and Baker (1994) have reared spat of *Pinctada maxima* at densities of 4 and 25 individuals /100cm^2 in downwellers feeding them with Tahitian *Isochrysis galbana*, *Chaetoceros calcitrans, C. gracilis, Nannochloropsis oculata* and *Tetraselmis chuii* twice daily at 40-285 cells/μl over 5 months and a growth rate of 9.6 mm and 6 mm/month shell height, has been observed in the two stocking densities respectively. Spat reared in plastic cages in sea at densities of 3 and 7 individuals/100 cm^2 exhibited a growth rate of 9.2 and 7.3 mm/ month respectively. Mortality five months after settlement has been 1-2% for those reared in hatchery and 9-10% for those reared in the sea.

Southgate and Beer (1997) have reported the successful production of *P. margaritifera* seed in Australia and evaluated hatchery and nursery techniques. Spat held in settlement tanks had a mean (± SE) dorso ventral shell height (DVH) of 1.38 (± 0.03) mm at 43 days of post fertilization when they were placed in plastic mesh trays and transferred to the sea. At 106 days of age, spat have been removed from collectors and graded. The mean (± SE) DVH of 106 day old spat has been 11.2 (± 2.7) mm, the largest individual had a DVH of 23 mm, with the smallest less than 2 mm. At grading, 0.2, 8.9 and 67.3% spat have been found to be retained on 15, 10 and 5 mm plastic mesh respectively, and 23.6% falling through the 5 mm mesh. DVH has been significantly higher in pearl oysters held in plastic trays at a density of 100 per tray (40.48 ± 0.9 mm). At this density the APM (39.68 ± 0.9 mm) and wet weight (7.44 ± 0.4g) have been found to be maximum. In pearl oysters held in pearl nets the DVH (39.22 ± 0.6 mm),

APM (38.36 ± 0.6 mm), hinge length (34.47 ± 0.5 mm) and wet weight (6.84 ± 0.8 g) have been observed to be highest at the lowest density of 20 per net. These parameters have been found differ significantly from those of juveniles held at a density of 50 per net. Growth of juveniles held at densities of 20 and 50 per net have been noted to be significantly higher than that of juveniles held at densities of 100, 150 and 200 per net. The presence of the leatherjacket, *Paramonacanthus japonicus* in culture trays and nets have been found to significantly affect growth rates of the pearl oyster spat.

Robert *et al.* (1993) compared the growth rate of the Japanese oyster, *Crassostrea gigas* stocked in cylinders and in traditional bags. Robert and Gerard (1990) found variation in growth rate of spat of the Pacific oyster, *C. gigas* and the scallop, *Pecten maximus*. *C. gigas* grew from 4 to 92 g. in 12 months with negligible mortality when stocked at the rate of 20 oysters in each pill shaped basket (Hughes-Games, 1977). Walne and Davis (1977) have studied the survival and growth rate of 50 oysters of *C. gigas* reared in trays. Wisely *et al.* (1979 a, b, c) have studied the growth rate of the Sydney rock oyster, *S. commercialis* at a stocking density of 450-500 oysters/tray. Tray size was 1.8 x 0.9m. They have observed that stocking density is a limiting factor for good growth. Growth rates of the spat of the rock oysters declined with increasing stocking density in sectionalized trays kept in intertidal zone (Holliday *et al.*, 1991). The efficiency of a nursery system is influenced by stocking densities and growth rates of individual oyster spat decrease with increasing stocking density, while overall spat production (weight/unit area) increases (Neudecker, 1981).

Chen (1984) attributes the increase in production of small abalones and hard clams in Taiwan to many factors including low stocking density (250 individual/m^2). The nursery culture of young spat remains the main constraint in increasing the hatchery productivity in French Polynesia (Coeroli *et al.*, 1984). Crawford *et al.* (1988) have assessed four culture methods in the ocean nursery rearing of the giant clam, *Tridacna gigas* and observed maximum growth in the

intertidal benthic method at a stocking density of 138 clams/m^2. Rearing at different densities of *T. gigas* juvenile for 5 months also have shown significant differences in length (Crawford *et al.*, 1986). Monteforte and Morales (2000) have studied the growth and survival of *P. margaritifera* at stocking density of 132-148 individual/ m^2 in Baja California, Mexico. In intensive rearing systems the stocking densities of oysters may be as high as 120,000-individual/m^2 (Bacher and Baud, 1992). Manzi *et al.* (1986) have evaluated the growth of seed clams, *Mercenaria mercenaria* in upflow nursery system at various densities ranging from 2.5 to 40.0 kg/ m^2.

The earlier experiments on maintaining the pearl oyster under tank conditions although of very preliminary nature was carried out by Kuwatani and others in Japan (Kuwatani and Nishi, 1969; Kuwatani *et al.*, 1969 and Kuwatani *et al.*, 1974). They monitored the effect of nitrate and the growth of the Japanese pearl oyster, *P. fucata.* They concluded that nitrate less than 10,000 μg atom nitrogen / lit. in rearing water has no harmful influences on the growth of oysters. They also studied the effect of number of animals and the exchange of water with fresh sea water on the growth of pearl oyster. They observed that a combination of factors such as pH, alkalinity, number of animals and exchange rate of water influenced the growth of oysters. Kuwatani *et al.* (1974) conducted experiments in tanks on oysters fed with a diet of rice powder. They found that the oysters gained more weight in tanks with high water temperature i.e. 30°C than in the tanks with low temperature i.e. 14°C.

The Central Marine Fisheries Research Institute (CMFRI) attempted the development of a technology for cultured pearl in onshore tanks (Rao and Devaraj, 1996). The results have shown that onshore pearl culture is less risky and more economical when compared to open sea pearl culture. It provides an opportunity to combine all key environmental factors together at the optimum level through good planning and management to make pearl culture highly successful.

Onshore culture systems for bivalves have been developed at Woods Hole, U.S.A. (Ryther, 1971; Ryther *et al.*, 1972) and at St. Croix, U.S. Virgin Islands (Roels *et al.*, 1978). These systems have been developed to utilize secondary treated sewage effluent as a nutrient source in culturing phytoplankters to feed commercially important bivalves. Pruder *et al.* (1977) describe the hatchery techniques for a controlled environment- molluscan mariculture system. A prognosis of shellfish mariculture in controlled environment has been presented by Epifanio *et al.* (1975), who have stated that the rate of consumption of food, oxygen and dissolved chemicals by the animal, rate of production of wastes and tolerance of the bivalves to various water quality conditions are of paramount importance.

A review of the literature on the optimum density in bivalve culture reveals that density will depend on species, size, type of culture and food requirements of spat. Although a wealth of information is available on the stocking densities in the sea based culture system, there is very little information on onshore practices. The present study was designed to identify the optimum stocking density of *P. fucata* spat in onshore culture.

2. 2. ALGAL CONCENTRATION AND NUTRITIVE VALUE OF MICROALGAE

Considerable research has been conducted on rearing of larvae and spat of oysters (Bruce *et al.*, 1940, Loosanoff and Davis, 1963, Walne, 1964, Alagarswami *et al.*, 1983, 1989) to determine the microalgae which are most suitable for seed production in hatchery. The common algal species used for raring oyster spat are *Isochrysis* sp., *Chaetoceros* sp., *Tetraselmis* sp. and *Skeletonema* sp. *I. galbana* is the species historically first used as a live food in molluscan mariculture (Bruce *et al.*, 1940). The small size and lack of cell wall render these cells ingestible by small, larval invertebrates and easily digested (Walne, 1964; Ukeles, 1975;). Commercially important oysters are farmed under natural conditions in coastal waters (Nell, 1993) and grow on diverse natural

diets, which vary depending on season, location and environmental conditions (Nell, 1992). However, in hatcheries algal diets have been used since the 1940s (Bruce *et al*, 1940). The dependence upon the various algal species for feed during the hatchery and nursery phases has caused problems due to high cost and unpredictable supply. This has prompted a search for non-algal food materials; however, to date no satisfactory non-algal diet is available for commercial bivalve culture (Urban and Langdon, 1984). Similarly, food cost has been a major limitation to the commercial development of intensive controlled oyster-culture (Epifanio *et al.*, 1975; Claus, 1981).

Early attempts to find algal substitutes involved the development of commercial oyster fattening diets based on cereal starch (Haven, 1965; Turgeon and Haven, 1978; Nell and Wisely, 1983, 1984); however, limitations in the understanding of nutritional requirements have hampered efforts. Recent advances in the development of encapsulated diets (Langdon, 1989; Langdon and DeBevoise, 1990; Southgate *et al.*, 1992) have indicated a more precise assessment of the nutrient requirements of oysters than it has been possible earlier (Nell, 1992).

Martin and Mengus (1977) and Douillet (1989) have shown that successful supplementation of algal diets with the inclusion of pure or mixed cultures of microbes has potential for significantly reducing the cost of live food diets for oysters.

Natural foods

The diet of oysters in the wild

In Pulicat Lake, South India, the stomach contents of the oyster, *Crassostrea madrasensis* have been reported to consist of 52.8% diatoms, 45.7% detritus and 1.5% zooplankton (Thangavelu, 1988). The proportion of detritus in the diet ranged from 28.0 to 64.6% over the two-year study. In the oyster beds of Chesapeake Bay, Delaware, Van Valkenburg *et al.* (1978) have

recorded that detritus form 77% of the seston. The percentages of seston in subestuaries of the Choptank Tiver, Maryland have been found to be highest in summer (Berg and Newell, 1986).

The amount of cellulose in the carbohydrate fraction of detritus depends on its origin (Langdon and Newell, 1990). In Canary creek, Delaware, which drains extensive marshland dominated by the marsh grass, *Spartina alterniflora* (Loisel) the carbohydrate fraction has been stated from 2 to 43% (Kreeger, 1986; Langdon and Newell, 1990) and may contain 85% cellulose (Newell and Langdon, 1986). The cellulose portion of the carbohydrate fraction has been found to be largely unavailable to *Crassostrea virginica* (Gmelin) (Crosby *et al.*, 1989; Langdon and Newell, 1990). Stuart *et al.* (1982) have demonstrated that the ribbed mussel, *Aulacomya ater* (Molina) could digest and assimilate upto 50% of detritus prepared from kelp. Detritus from such macroalgae is more readily digestible than detritus from the angiosperm, *S. alterniflora* (Findlay and Tenore, 1982).

Bacteria are another natural food source for oysters. They may be present free (unattached) or, more commonly, attached to detritus microalgae or zooplankton. The oyster, *Crassostrea virginica* filters free bacteria with an efficiency of only 5% (Langdon and Newell, 1990). However, it was estimated with the use of ^{14}C and ^{15}N labelled bacteria, that attached and unattached bacteria could contribute 5.5% of the metabolic carbon requirement and 27% of the nitrogen requirements of *C. virginica* in Canary creek, Delaware (Langdon and Newell, 1990). The high concentrations of attached bacteria in Chesapeake Bay were found to contribute upto 19.2% of the total carbon and to make a significant contribution to the nitrogen requirement of *C. virginica* in this estuary (Crosby and Newell, 1990). Although few studies on protozoans as a food source for oysters have been conducted, it appears that they may also form an important component of the natural diet of oysters (Baldwin and Newell, 1991).

Oyster diet consists of other possible items apart from microalgae. The amount of food available in estuaries for bivalves may be assessed by such measurements of seston, consisting of living and non-living particles including phytoplankton, zooplankton, dead organisms, organic detritus and particulate minerals, as particulate organic nitrogen (PON) (Wilson, 1987). The ratio of POM to particulate inorganic matter (PIM) in the seston may also be important, because a high PIM concentration could dilute ingested diet (Widdows *et al.*, 1979). The presence of a PIM such as kaolin could improve ingestion and/or digestion rates and/or efficiencies (Langdon and Seigfried, 1984). A food index defined as the percentage of food (Food = lipid + carbohydrate + protein) in the total seston was used as a measurement of available food by Soniat and Ray (1985). Chlorophyll a is another measurement often taken (Brown and Hartwick, 1988a,b), but it indicates the abundance of phytoplankton only (Jeffrey, 1981).

Physical aspects of nutrition

Particle size

The feeding mechanism in oysters depends on the ciliary action of gills driving a current of water through the ostia (Ukeles, 1969). During passage through the gills, particulate matter is filtered out, trapped in mucus, and transported to the labial palps, where it is ingested, or, if it is large or spiny, rejected (Ukeles, 1969). As oysters ingest both algae and non-nutritive particles such as graphite (Owen, 1966), selection appears to be made on particle size (Wisely and Reid, 1978) and possibly other factors such as chemical acceptability (Ukeles, 1969). Both filtration efficiency and ingestion are affected by particle size. Owen (1966) has observed that *Crassostrea virginica* takes diatoms (4-24μm), and allows 70-80% of *Escherichia coli* (1-2μm) to pass. Similarly, adult *C. virginica* has been found to filter out particles in the range of 3-12μm 30-50% more efficiently than those in the range of 1-3μm (Haven and Morales-Alamo, 1970).

CONCENTRATION

The role of silt/kaolin

The blue mussel, *Mytilus edulis* L., clearly benefits from small quantities of suspended silt in the water (Winter, 1978), as silt assists in grinding up of food in the digestive tract (Murken, 1976), and also because the organic matter component of natural silt is an important source of nutrients (Kiorboe *et al.*, 1981).

The value of totally inorganic kaolin supplements in artificial diets for oyster spat is not so clear. Urban and Langdon (1984) have reported increased growth in *C. virginica* when a diet of algae and yeast was supplemented with kaolin at 20 mg L^{-1}, growth was comparable to that of spat fed only algae. However, kaolin did not improve growth of spat fed with artificial diets other than yeast. Kaolin suspensions have been recommended to improve the artificial diets to oysters (Langdon and Bolton, 1984; Langdon and Siegfried, 1984) and of diets with algal contents (Ewart and Carriker, 1983). Sornin *et al.* (1988) have improved the retention of particles in the digestive tract of Pacific oysters, *Crassostrea gigas* (Thunberg), by the addition of kaolin particles of between 3 and 4μm in size. The benefits of kaolin in the diet of oysters accrue both from its assistance in grinding up food in oyster's digestive tract and from the nutrients obtained from bacteria growing on kaolin.

Hidu *et al.* (1981) and Utting and Spencer (1991) have reared cultchless spat of European and American oysters in closely controlled environmental conditions in a hatchery up to 2-3 mm in size. Hickman *et al.* (1999) have opined that nursery rearing of oyster spat in hatchery upwellers follows the hatchery processes of spawning, larval rearing and settlement or metamorphosis and it precedes the final grow-out phase.

Maximum food concentration

Based on maximum glycogen content, condition index and meat weight gain, Nell and Wisely (1984) have recommended 9 mg of dietary particles L^{-1} of sea water for the fattening of Sydney rock oyster, *Saccostrea commercialis*, considerably more than the 2 mg L^{-1} of starch, estimated by the production of a minimum ratio of pseudofaeces to faeces, as reported by Wisely and Reid (1978), suggesting that for rapid oyster fattening or maximum meat weight of *S. commercialis* some food may need to be wasted as pseudofaeces. Based on the weight gain and glycogen content of the American oyster, *C. virginica*, Turgeon and Haven (1978) have recommended 5 mg L^{-1} of starch to improve meat condition in adult oysters. Based on pseudofaeces production, Epifanio and Ewart (1977) have recommended a maximum concentration of live algae at 10 mg dry weight L^{-1}.

Although food concentration can affect the rate of filtration in molluscs, studies of the blue mussel, *M. edulis* show that, despite decreasing filtration with increasing food concentrations, the actual ingestion rate is not affected; that is, a similar amount of food is consumed (Winter 1973, 1978).

Feeding rate

Feeding rate, the daily amount of food supplied per oyster, is an important management tool for conditioning (gonad maturation) of broodstock, for spawning, for "fattening" or increasing meat size for marketing and for rearing larvae in hatcheries and spat in nurseries. The suggested feeding rates below emphasise maximum food consumption and not the highest assimilation efficiency.

An empirical equation for the maximum daily ration for *C. virginica* of any size at 20°C has been evolved by Epifanio and Ewart (1977):

$$QR=0.01w^{-0.41}$$

It was refined by Epifanio (1979) to:

$$QR=0.01w^{-0.33},$$

Where QR is daily dry weight (g) of ration g^{-1} live weight of oyster, and w is live weight of oyster (g). The exponent in the latter equation is closer to the theoretical value used by Pruder *et al.*, (1977). Both equations are suitable for both live algal diets and artificial diets, although they may underestimate maximum food consumption at high (28°C) temperatures (Epifanio, 1983).

A similar equation fo *C. virginica* has been given by Pruder *et al.* (1976), using wild collected adults and laboratory-reared spat:

$$Y = 5.3\ w^{-0.21},$$

Where Y is the daily ration of algal cells x 10^8 g^{-1} live weight of oyster, and w is the live weight of oyster (g) (Pruder *et al.*, 1977). Pruder *et al.* (1976, 1977) have used a 50/50 (1:1) mixture (by cell number) of *Thalassiosira pseudonana* Hasle & Heimdal and *Isochrysis galbana* Green. These equations could also be used for artificial diets by converting the daily ration of algal cell numbers to dry weight (g).

Urban *et al.* (1983), however, have reported that all these formulae for calculating adequate rations for *C. virginica* may not be sufficient for maximum growth of juvenile oysters. They recommended an effective daily ration (dry weight) of 2.8% of the oyster live weight. Whatever method is used for calculating the daily ration for small, rapidly growing oysters, it would have to be recalculated weekly. As daily ration depends on culture conditions, all methods for calculating should only be used as a guide by aquaculturists.

The role of calcium and the processes involved in shell formation and regeneration in molluscs have been reviewed by several authors (Wilbur, 1964, 1972; Timmermans, 1969; Sick and Siegfried, 1983). However, increases of growth in shell and meat in oysters do not always occur simultaneously (Brown and Hartwick, 1988a,b). Hilbish (1986) has mentioned that great care should be

taken when interpreting experimental results. Epifanio (1983) has stated that both food availability and temperature affect the patterns of shell deposition and meat growth in oysters. Under conditions of long-term low food availability, shell thickening is more energetically efficient than meat growth (Brown and Hartwick, 1988b). Marked reduction in the calcium concentration of sea water may reduce or prevent calcification in oysters (Kado, 1960; Conger *et al.*, 1978). Shell growth of *C. virginica* stops at calcium concentration of about 60 mg L^{-1} of sea water or less (Conger *et al.*, 1978). Calcium concentrations in water can drop during periods of low salinity (Nell and Holliday, 1988), which could result in stress and reduction in both meat weight gain and shell deposition in oysters. Kado (1960) has reported that when the calcium concentration of sea water increased from about 124 mg L^{-1} to the normal concentration of about 400 mg L^{-1}, the rate of calcium deposition in *C. gigas* increased, but did not increase further at higher concentrations.

Ward and MacDonald (1996) have examined the pre-ingestive feeding responses of two species of subtropical bivalves, the turkey wing, *Arca zebra* (Swainson) and the Atlantic pearl oyster, *Pinctada imbricata* Roeding, to an acute increase in natural suspended particulate matter concentration. These two sympatric species inhabit coastal regions of Bermuda and possess fundamentally different gill structures. Their simulated resuspension activity has been found to cause a four-fold increase in the concentration of suspended particles, feeding rates and a significant increase in the rate of pseudofaeces production in both species. *P. imbricata* indiscriminately rejected suspended particulate matter in the form of pseudofaeces. Specimens of *A. zebra*, however, demonstrated particle selection, rejecting material with significantly higher carbon and lower nitrogen concentrations, thereby increasing the quality of material ingested by approximately 31%.

The inter-relationship between food availability and feeding response of the aquaculture species have been studied by Stenton-Dozey and Brown (1994); Bacon *et al.* (1998) and Mac Donald *et al.* (1998). Wong and Cheung (2001)

have studied the physiological parameters of feeding in *P. viridis* which included clearance rate (CR), absorption rate (AR) and absorption efficiency (AE), and their relationships with food availability in the experimental water column at Kat O, the site with the fastest growth recorded for *P. viridis* in Hong Kong.

Widdows (1978a), Bayne and Worall (1980), Sprung (1984) and MacDonald and Thompson (1985) have stated that food availability and temperature are important environmental factors influencing the growth of bivlaves. Numaguchi (1995) has opined that a protracted period of unfed condition may cause more severe wasting and higher mortalities of pearl oysters. However, Rio-Portilla *et al.* (1992) have opined that food concentration, but not temperature had an important influence on condition index in *Pteria sterna*. Coutteau *et al.* (1994) have cultured juveniles of the hard clam (1 mg live weight) in an 18 l recirculating system for 2 to 3 weeks and found the optimal weight-specific daily ration for a mixture (50/50 on dry weight basis) of *Isochrysis galbana* (clone T-ISO) and *Chaetoceros gracilis* is 1.5 to 2% dry weight per wet weight of seed.

GROWTH

Rao and Devaraj (1996) have developed technology for producing marine pearls in onshore tanks like any other pond system under controlled conditions. At Kakinada, Andhra Pradesh, India, young ones of the pearl oyster, *Pinctada fucata* ranging from 10 to 19 mm in DVM with a mean value of 15 mm reared in cement tanks in onshore system grew to a mean size (DVM) of 37.7 mm with a range of 31-44 mm at the end of 58 days. The pearl oyster grew to a size of 48.3 mm at the end of 113 days. The oysters in cement tanks were fed with *Chaetoceros* and *Isochrysis*.

The pearl production technology developed by CMFRI in 1973 at Tuticorin has been refined and upgraded for mass scale commercial production in the Gulf of Mannar on the east coast of India (Alagarswami 1987b). Alagarswami *et.al.*

(1983) observed growth from 0.37 mm to 12.0 mm over a growth period of 3 months. Chellam (1988) found the growth of pearl oyster at Tuticorin to be in the size of 3.0 – 11.2 mm, when they are 30 days old. At the end of 2nd month they attain a length range of 9.0 to 17.8 mm and reached a size of 22.0 to 35.2 mm in a 5 month period. Appukuttan *et al.* (1998) have studied spat growth of *Pinctada fucata* transported from Tuticorin and reared at Andhakaranazhi, a village 50 km south of Cochin, Kerala, India. The spat with an average size of 15.9 mm exhibited growth to an average size of 46.2 mm in five months. At Calicut, Kerala, India, pearl oyster spat with an average size of 17.7 mm grew to 43 mm in 5 months with a growth rate of 5 mm/month (Appukuttan *et al.*, 1998).

Maguire and Burnell (2001) have studied the growth rate of scallop, *Pecten maximus* spat cultured in lantern nets and opined that spat cultured in lantern nets had a significantly higher growth rate than those cultured in pearl nets and spat cultured at low densities had a higher growth rate and carbohydrate content during the summer than those reared at high densities.

Nayar and Al-Rumaidh (1993) have studied the rate of growth of spat and yearlings of the pearl oyster, *Pinctada radiata* (Leach) of Bahrain waters and opined that spat grows to a size of about 45mm at the end of one year and 70 mm in two years. Numaguchi (1994) has estimated the mean growth rates in whole weight of one and two year old pearl oyster, *Pinctada fucata martensii* in Ohmura Bay to be 138-157 mg/day and 68-69 mg/day, respectively.

Many short-term studies have focussed on the effect of the food level on growth in bivalves (Widdows, 1978a, b; Griffiths, 1980; Navarro and Winter, 1982; Winter *et al.*, 1984; Winter, 1978; Bayne and Newell, 1983; Hawkins and Bayne, 1990).

Rose and Baker (1994) have reared spat of *Pinctada maxima* at densities of 4 and 25 individuals /100cm^2 in downwellers feeding them with Tahitian *Isochrysis galbana*, *Chaetoceros calcitrans*, *C. gracilis*, *Nannochloropsis oculata*

and *Tetraselmis chuii* twice daily at 40-285 cells/μl over 5 months and a growth rate of 9.6 mm and 6 mm/month shell height, has been observed in the two stocking densities respectively. Spat reared in plastic cages in sea at densities of 3 and 7 individuals/100 cm^2 exhibited a growth rate of 9.2 and 7.3 mm/ month respectively. Mortality five months after settlement has been 1-2% for those reared in hatchery and 9-10% for those reared in the sea.

Nalluchinnappan *et al.* (1982) have reared the pearl oyster, *Pinctada fucata* in pigeonhole box cages and noted that in the smaller size group, the average growth in dorsoventral measurement has been 9.9 to 24.9 mm in seven months.

Robert *et al.* (1993) have studied the growth of spat and 18 month old *Crassostrea gigas* oysters, reared in PVC meshed cylinders and in traditional bags in Bay of Arcachon, France and found that the growth in height and in whole weight has been lower in cylinders than in bags but the quality of meat and shell was better and carbohydrate content higher.

The stocking density, type of culture system, abundance and composition of associated species, hydrographic parameters prevailing in different culture locations, and depth at which culture systems are installed, are among the many important factors determining the culture strategy during juvenile and adult stages, as well as during pearl formation (Gervis and Sims, 1992; Monteforte, 1996; Taylor *et al.*, 1997a; Taylor *et al.*, 1997b; Taylor *et al.*, 1998). These factors influence the growth rate, shell shape, survival and pearl quality (Monteforte *et al.*, 1994; Monteforte, 1995, 1996; Taylor *et al.*, 1997a, b; Taylor *et al.*, 1998; Chellam, 1978; Chellam *et al.*, 1987; Chellam *et al.*, 1987; Monteforte and Morales-Mulia, 2000).

Southgate and Beer (1997) have reported the successful production of *P. margaritifera* seed in Australia and evaluated hatchery and nursery techniques. Spat held in settlement tanks had a mean (± SE) dorso ventral shell

height (DVH) of 1.38 (± 0.03) mm at 43 days of post fertilization when they were placed in plastic mesh trays and transferred to the sea. At 106 days of age, spat have been removed from collectors and graded. The mean (± SE) DVH of 106 day old spat has been 11.2 (± 2.7) mm, the largest individual had a DVH of 23 mm, with the smallest less than 2 mm. At grading, 0.2, 8.9 and 67.3% spat have been found to be retained on 15, 10 and 5 mm plastic mesh respectively, and 23.6% falling through the 5 mm mesh. DVH has been significantly higher in pearl oysters held in plastic trays at a density of 100 per tray (40.48 ± 0.9 mm). At this density the APM (39.68 ± 0.9 mm) and wet weight (7.44 ± 0.4g) have been found to be maximum. In pearl oysters held in pearl nets the DVH (39.22 ± 0.6 mm), APM (38.36 ± 0.6 mm), hinge length (34.47 ± 0.5 mm) and wet weight (6.84 ± 0.8 g) have been observed to be highest at the lowest density of 20 per net. These parameters have been found not differ significantly from those of juveniles held at a density of 50 per net. Growth of juveniles held at densities of 20 and 50 per net have been noted to be significantly higher than that of juveniles held at densities of 100, 150 and 200 per net. The presence of the leatherjacket, *Paramonacanthus japonicus* in culture trays and nets have been found to significantly affect growth rates of the pearl oyster spat.

Mallet and Haley (1983), Dickie *et al.* (1984) and Mason *et al.* (1998) have observed that rate of growth of bivalve molluscs are often extremely variable between individuals of the same age. Grant (1996) and Bayne *et al.* (1999a) have stated that high levels of variations in the underlying physiological processes contribute to this.

Petri and Vareschi (1997) have studied the growth of young mussels utilizing different food algae *Phaeocystis globosa* colonies, *P. globosa* single cells, the diatom *Phaeodactylum tricornutum*, the green alga *Dunaliella salina*, and artificial food.

FEEDING

Frequency of feeding

A tidal rhythm for digestion in the flat oyster, *O. edulis* has been recorded by Morton (1971, 1977). Langton and Gabbot (1974) have stated that this is not to be endogenous but controlled by feeding activity. Higgins (1980a) has mentioned that although continuously fed oysters filter most of the time, the rates at which continuously and intermittently fed *C. virginica* ingested food (consumption per hour) has been found not different; consequently the daily ration taken by intermittently fed *C. virginica* is much less than that of oysters fed continuously at the same algal concentration (Higgins, 1980b). Intertidal bivalves, which have a shorter time available for feeding, do not compensate by increasing their feeding rate during immersion (Bayne and Hawkins, 1988). However, Langton and McKay (1974; 1976) have reported that *C. gigas* spat in the laboratory grow faster when fed intermittently (6h fed/6h unfed) than when fed continuously with the same number of algal cells per day, a phenomenon that could be put to good use in hatcheries.

NUTRITIONAL VALUE

Algal Diets

Algal suspensions

A number of studies have been conducted which assess the value of algal species as a food source for oysters and their larvae. After testing five algal species singly and in various combinations, Laing and Millican (1986) have recommended the diatom, *Chaetoceros calcitrans* (Paulsen) Takano as a single species diet for *C. virginica* spat. Walne (1970) has tested nineteen algal species as food for juvenile flat oysters (*Ostrea edulis* L.) and found *P. lutheri* and *C. calcitrans* to be among the best. *P. lutheri* has high concentrations of both the essential 20:5ω-3 and 22:6ω-3 fatty acids (Brown *et al.*, 1989). These two fatty

acids individually or in combination have been found to be important for growth in *O. edulis* spat (Enright *et al.*, 1986a, 1986b) and Pacific oyster, *Crassostrea gigas* (Thunberg) spat (Langdon and Waldock, 1981). Maximum growth was observed in spat of *S. commercialis* when fed with the diatom, *Skeletonema dostatum* (Greville) Cleve (O'Connor, *et al.*, 1992). From similarities in protein levels (Enright *et al.*, 1986a) and protein aminoacid compositions of the algal species (Webb and Chu, 1983) it has been suggested that protein compositions alone are unlikely to be important factors in determining the food value of algal species for oysters.

It is considered that both the quality and quantity of seston available to mussels vary both on tidal and seasonal time scales, and are major factors influencing growth rate (Kiorboe and Mohlenberg, 1981; Shumway *et al.*, 1985; Bayne *et al.*, 1987, 1989, 1993; Lucas *et al.*, 1987; Newell *et al.*, 1989; Hawkins and Bayne, 1991; Navarro *et al.*, 1991; Hawkins and Bayne, 1992; Newell and Shumway, 1993). Hawkins *et al.* (1997) have investigated short-term responses of *Mytilus edulis* L. to experimental changes in the amount and composition of suspended seston. They have studied feeding behavior over ranges of food availability and quality that extend well beyond earlier limits and considered that mussels have ability to selectively reject inorganic particles as pseudofaeces, thereby enriching the organic content of ingested matter by 30% more than the organic content of natural filtered seston.

Algal culture management is a costly process in bivalve seed production (De Pauw, 1981). Generally, bivalve molluscs fed on diets consisting of more than one species of algae grow faster than those fed on single species diets. Moreover, certain algal species considered as 'poor' diets are better utilized in growth when fed in combination with other species of algae (Romberger and Epifanio, 1981). Fidalgo *et al.* (1994) have reported that diets consisting of more than one algal species generally promote more rapid growth than diets consisting of a single species. Certain algal species, which do not support growth when fed

as a single species diet, are better utilized for growth when fed in combination with other species (Laing and Verdugo, 1991). Albentosa *et al.* (1993) have examined the nutritional value of two microalgal species, *Isochrysis galbana* and *Tetraselmis suecica*, in the spat of bivalve *Venerupis pullastra*. Their study included the effect of single species diets and mixture of two species diet on spat ingestion rates, growth and biochemical composition. Mixed algal diets are regularly used in many bivalve hatcheries (Napolitano *et al.*, 1990), wherein the general practice is to culture each species separately and then feed them in combination.

The Pacific oyster, *Crassostrea gigas* production in many countries relies on hatcheries and nurseries, which grow larvae and juveniles (Chew, 1990; Dix, 1991; Donaldson, 1991). Larvae and newly set spat are grown in hatcheries on a diet of cultured microalgae, commonly *Isochrysis* sp. (strain T. ISO), *Pavlova lutheri* and the diatoms *Chaetoceros calcitrans* and *Thalassiosira pseudonana* (Alagarswami *et al.*, 1989; Krishnan and Alagarswami, 1993; Coutteau and Sorgeloos, 1992). When juveniles are of a size range of ~500 μm to 2.5 μm, they are typically transferred to tanks and sea water is pumped through continuously from an estuary or bay (Rodhouse *et al.*, 1981).

Phatarpekar *et al.* (2000) have studied the growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures. Okauchi (1990) has estimated food value of *Isochrysis* aff. *galbana* (clone T-ISO) for pearl oyster *Pinctada fucata* spat and compared it with those of *I. galbana* and *Chaetoceros gracilis*. Albentosa *et al.* (1997) have observed that the nutritional value of different algal diets, selected on the basis of their specific fatty acid composition, is, apart from lipids, affected by several other factors, e.g., assimilation efficiency and biochemical composition in the clam, *Ruditapes decussatus*.

The nutritional value of a given species of microalgae depends on the nature and composition of its biochemical constituents (Whyte *et al.*, 1990) and

its physical suitability (Rose and Baker, 1994). There is considerable variability in the ability of different algal species to support adequate growth of bivalves (Brown and Jeffrey, 1992). There are a number of reports on the rearing of *P. maxima* spat, (Tanaka and Kumeta, 1981; Rose, 1990; Rose and Baker, 1994) and nutritive value of microalgal species for spat of the Pacific oyster, *Crassostrea gigas* (Langdon and Waldock, 1981; Laing and Verdugo, 1991; Laing and Millican, 1992; Thompson *et al*, 1993), the European flat oyster *Ostrea edulis* (Walne, 1963, 1970; Laing and Verdugo, 1991), the Sydney rock oyster *Saccostrea commercialis* (O' Connor *et al.*, 1992), the clams *Mercenaria mercenaria* (Laing and Verdugo, 1991; Wikfors *et al.*, 1992) and *Tapes philipinarum* (Laing and Millican, 1991; Laing and Verdugo, 1991), the mussel *Mytilus galloprovincialis* (Fidalgo *et al.*, 1994) and the scallops *Patinopecten yessoensis* (Whyte *et al.*, 1989) and *Crassoidoma gigantea* (Whyte *et al.*, 1990).

Taylor *et al.* (1997) have assessed five species of microlagae the golden brown flagellates *Isochrysis* aff. *galbana* (clone T-ISO) and *Pavlova lutheri*, the diatoms *Chaetoceros muelleri* and *C. calcitrans* and the green flagellate *Tetraselmis suecica* for their nutritional value for the pearl oyster, *P. maxima* spat and observed that pearl oyster spat fed with *Chaetoceros muelleri* showed lowest organic content. T-ISO and *C. muelleri* have been considered by Jeffrey *et al.* (1992) as good sub-tropical and tropical species, *P. lutheri* as a good sub-tropical species and *C. calcitrans* and *T. suecica* as excellent universal species as feed for bivalve spat.

The relationship between the rates of ingestion and of efficiencies of absorption of energy from ingested food varies with the nutritional quality of the ration, resulting in changes to net energy gain that are disproportionate to the change in dietary quality alone (Navarro *et al.*, 1994; Hawkins *et al.*, 1996; Arifin and Bendell-Young, 1997). Wieser (1989) has drawn attention to a different aspect of physiological compensation, in which high rates of growth are achieved by a change in patterns of energy allocation between competing physiological

demands. Compensatory allocations of energy to support growth result in changes in the apparent metabolic costs of component processes (Rombough, 1994; Wieser, 1994). Bayne (2000) has reported that fast growth is associated with faster rates of feeding, reduced metabolic rates and lower metabolic costs of growth.

CULTURE

Lucas (1994) has investigated the culture of juveniles of gold lip oyster, *Pinctada maxima*, in small recirculating systems, and their nutritional requirements, as a means to improving the management of hatchery produced spat.

Soletchnik *et al.* (2001) have studied a commercial level production of algae using an enrichment technique developed for intensive aquaculture (Baud *et al.*, 1995; Hussenot, 1992; Hussenot and Gautier, 1994; Hussenot *et al.*, 1998; Gautier *et al.*, 1993) and measured the effect of dietary supplement during fattening of the *C. gigas* in oyster ponds. Most hatcheries either grow oyster spat in land-based systems of flowing, unfiltered sea water (Ingle *et al.*, 1981) or in natural waters, often in trays or other containers (Paynter and Dimichelle, 1990).

2. 3. BIOCHEMICAL COMPOSITION OF MICROALGAE AND SPAT

Difficulty has been encountered in determining the nutrient requirements of oysters because of the need to first meet the desirable physical characteristics of a filter-feeder diet (Nell, 1992). However, the development of diets packed in appropriately sized microcapsules has eased such oyster studies. Nell (1992) has stated that the protein requirement of an oyster is approximately 20% of the dryweight of the diet and the total lipid requirement probably not more than 5%. Oysters have a small but specific requirement for the long-chain fatty acids 20:5 ω-3 and 22:6 ω -3, with the latter being of more importance (Nell, 1992). Other factors such as dietary carbohydrate contribute to the nutritional value of an alga

to bivalves (Enright *et al.*, 1986b; Whyte *et al.*, 1990). Very little is known about the essential and optimum aminoacid requirements in food protein for oysters, nor about their vitamin requirements. However, mineral requirements of oysters are probably largely met by direct absorption from sea water (Nell, 1992) and food.

The role of dissolved organic nutrients

The role of dissolved organic nutrients in the diet of oysters has received much attention. In filter-feeding, the large epithelial surface areas of the gill and mantle in oysters are exposed to a large water volume; consequently these surfaces are ideal for the direct absorption of nutrients. Pequignat (1973) has demonstrated that in adult bivalves, the gill is the main organ responsible for the uptake of small organic molecules from sea water, whereas in veligers the velum is the major site of uptake for dissolved organic matter (Manahan and Crisp, 1982). Many other studies have focussed on the role of dissolved aminoacids in the nutrition of marine bivalves (Stewart, 1979; Stephens, 1981,1983; Manahan and Crisp, 1982, Wright, 1982; Manahan, 1990).

Oysters have the ability to absorb aminoacids (Rice *et al.*, 1980; Manahan, 1983b, 1990; Nell *et al.*, 1983; Nell and Dunkley, 1984; Nell and Gibbs, 1986), vitamins (Nell *et al.*, 1983), fatty acids (Bunde and Fried, 1978) and glucose (Schulte *et al.*, 1973; Nell *et al.*, 1983; Melaouah, 1989; Welborn and Manahan, 1990) directly from sea water. Amino acids in particular are absorbed as early as the fertilized egg stage (Manahan, 1983a). Aminoacids taken up from solution are rapidly distributed to the internal tissues (Rice and Stephens, 1987).

It has been suggested that dissolved vitamins also have nutritional importance for oysters (Davis and Chanley, 1956; Urban and Langdon, 1984; Ukeles and Wikfors, 1988). To date, little is known about the benefits to oysters of the direct absorption of fatty acids (Stewart, 1979). The nutritional importance of glucose for bivalves have been well studied (Gillespie *et al.*, 1964; Welborn and Manahan, 1990).

Nutrient requirements

Protein, lipids, carbohydrates and vitamins

The protein requirement for the maximum growth of *C. virginica* spat fed on algae has been stated by Flaak and Epifanio (1978) to be 21% of the dry weight of the algae. The protein requirement for greatest meat weight gain of adult *S. commercialis* fed a microparticulate diet based on wheat starch and bacterial protein (Pruteen^{R}) has been approximately 17% of the dry weight of the diet (Nell and Wisely, 1983). Although mixtures of algae can provide a diet balanced in amino acids, the essential aminoacids required for oysters have not yet been determined. Langdon and Siegfried (1984) have shown that different protein sources in artificial diets produced different growth rates in *C. virginica* spat, which indicates that the protein sources vary in their ability to satisfy the aminoacid requirements of the oysters.

Castell and Trider (1974) have stated that the total lipid requirement of oysters is probably not more than 5% of the dry weight of the diet. Data on the growth and the tissue composition of oysters indicate that oysters have an essential fatty acid requirement for both the linolenic or (ω3) and linoleic or (ω6) series fatty acids (Trider and Castell, 1980).

Oyster diets should contain a high concentration of carbohydrates (Flaak and Epifanio, 1978; Wikfors *et al.*, 1984; Utting, 1986; Whyte *et al.*, 1990). The carbohydrate portion of the diet should contain a minimum of cellulose, as little is digested by oysters (Crosby *et al.*, 1989; Langdon and Newell, 1990). It is not clear whether oysters digest cellulose through the action of endogenous cellulase or with the help of bacteria in the digestive tract. A study of bacterial activity in *C. virginica* tissue has indicated that bacteria play a major role in the assimilation of cellulose (Crosby and Peele, 1987), whereas Newell and Langdon (1986) have found no difference in the very low cellulose assimilation of *C. virginica* treated

with antibiotics and of untreated controls. Brock *et al.* (1986) have demonstrated that cellulose activity of the hepatopancreas is a common phenomenon in natural oyster populations and later demonstrated that the intake of food high in cellulose, increases cellulolytic activity in the hepatopancreas of *C. gigas*. Brock (1989) has also shown that purified bacterial cellulases do digest cell walls of chlorophytes.

Although oysters can absorb vitamins directly from sea water (Nell *et al.*, 1983), spat of *C. virginica* fed microencapsulated vitamins have been found to grow faster than spat supplied with dissolved vitamins (Langdon, 1983b). Very little is known of the specific vitamin requirements of oysters, although a mixture of vitamins has been found to be required for maximum growth (Davis and Chanley, 1956; Urban and Langdon, 1984; Ukeles and Wikfors, 1988).

As with vitamins, oysters can directly absorb from sea water all the calcium which they require (Bevelander, 1952; Jordey, 1953; Sick *et al.*, 1979), as well as soluble phosphorus (Bevelander, 1952; Pomeroy and Haskin, 1954) and trace metals (Nell and Livanos, 1988; Nell and Chvojka, 1992).

Algae excrete organic substances termed ectocrines, which include sugars, aminoacids, fatty acids, vitamins, steroids and numerous other secondary metabolites (Lefevre, 1964; Stephens and Manahan, 1984). The ectocrines can affect filtration rates and particle selection in bivalves (Ward and Targett, 1989) and the nutritional value of algal diets (Manahan, 1983c).

Albentosa *et al.* (1996) have evaluated several monoalgal diets for the culture of the seed of *Ruditapes decussatus* using a method which can explain differing growth rates in relation to both physiological and biochemical parameters.

In five experiments, *C. gigas* and *O. edulis* juveniles receiving fertilized sea water have been found to be on an average 60% heavier after six weeks than oysters receiving unfertilized sea water (Spencer *et al.*, 1986). McCausland

et al. (1999) have assessed algal species as supplementary diets for juvenile Pacific oyster, *Crassostrea gigas* at a major nursery site in Australia. Choosing microalgae of different taxonomic classes, i.e., diatoms, prymnesiphytes, a cryptophyte and a chlorophyte with different biochemical compositions, including polyunsaturated fatty acids (PUFAs) and observed that supplemented feeding into *Dunaniella tertiolecta*, *C. calcitrans, Isochrysis* sp. (Strain T. ISO), an Australian strain of *P. pinguis*, *Pavlova* sp. and *Rhodomonas salina*; and algal pastes of *C. calcitrans* and *Skeletonema costatum* has given increase in growth rate of juveniles.

Chaetoceros is generally preferred over *Isochrysis* due to its nutritional superiority, particularly the higher content of highly unsaturated fatty acids (HUFA) (Napolitano *et al.*, 1990) and its ability for rapid growth. Lewin *et al.* (1979) have reported that the organic matter of *Chaetoceros armatum* is composed of 67.6% lipid, 29.7% protein, and 1.3% carbohydrate. Cultured diatoms of the species also have high levels of lipid and protein and less of carbohydrate. This diatom species is a major food source throughout the year for the Pacific razor clam, *Siliqua patula* along the Olympic peninsular coast. Protein constitute 47% and lipid 42% of the dry weight of the razor clam tissue. *Isochrysis* is a prolific grower, but is relatively low in HUFA content (Helm and Laing, 1987). Caers *et al.* (1999) have reported that the composition of the algal diet (*Dunaliella tertiolecta* and *Tetraselmis suecica*) as well as the lipid supplementaion level (20 and 40%), increased the growth and fatty acid composition of *Tapes philippinarum* spat.

Essential ω-3 fatty acids (20:5 and/or 22:6), and sterols (delta-5, except those with ethyl substitution on carbon-24) have shown the strongest correlation with spat growth (Wikfors *et al.*, 1991). Wikfors *et al.* (1996) have reported high sterol and essential fatty acid contents of several strains from the prasinophyte genus *Tetraselmis*, and demonstrated significantly more rapid spat growth than

growth with an equivalent ration of a standard hatchery diet (*Isochrysis* sp., strain T-ISO) or any unialgal diets tested.

Considering that the success of a molluscan hatchery, expressed in terms of mortality, growth or setting rate depends on the availability of suitable nutrients (Walne, 1970; Watanabe and Ackman, 1974; Webb and Chu, 1982; Enright *et al.*, 1986a; Whyte, 1987), knowledge of the biochemical composition of the phytoplankton supplied as food is essential in order to establish an adequate diet for bivalve larvae and juveniles (Fernandez-Reiriz *et al.*, 1989).

The specific nutrient requirements of molluscan larvae and spat are not completely known. While some authors pointed out the role of carbohydrates and lipids (Enright *et al.*, 1986b) as well as some polyunsaturated fatty acids (Helm *et al.*, 1973; Ackman, 1982; Langdon and Waldock, 1981; Langdon, 1982), other authors (Webb and Chu, 1982) opine that the importance of the food given to bivalve larvae and juveniles depends more on other nutrients, fatty acids, amino acids, monosaccharides, minerals and vitamins than the gross biochemical composition.

Although biochemical composition is not the only factor to be considered when studying the nutritional value of a phytoplanktonic species, other factors such as cell size, digestibility and even toxicity have been suggested as explanations for differences in the nutritional value among different species, knowledge of the biochemical composition of these species is essential in order to attain an optimal diet (Fernandez-Reiriz *et al.*, 1989).

A wide range of microalgae has been tested in bivalve seed rearing as not all species are equally successful in supporting growth of a particular animal (Davis and Guillard, 1958; Walne, 1970; Epifanio *et al.*, 1981; Enright *et al.*, 1986a). Factors such as the size, toxicity, digestibility and biochemical composition of the microalgae can account for the differences in their food values (Webb and Chu, 1983). It has also been established that the biochemical

composition of a given species of phytoplankton can be modified under different growing conditions (Webb and Chu, 1982; Fabregas *et al.*, 1985) and it also changes according to the growth phases of algae (Fernandez-Reiriz *et al.*, 1989).

Fernandez-Reiriz *et al.* (1983), Murado *et al.* (1985), Fabregas *et al.* (1985, 1986) and Utting (1985) have studied changes in the biochemical composition of phytoplankton during the different growth phases. Published data usually refer to a given moment of culture (Parsons *et al.*, 1961; Langdon and Waldock, 1981; Webb and Chu, 1982; Utting, 1985; Ben-Amotz *et al.*, 1985, 1987; Whyte, 1987). In the case of the fatty acid composition, most of the published data describe a particular point in the growth curve (Ackman *et al.*, 1968; Watanabe and Ackman, 1974; Langdon and Waldock, 1981; Webb and Chu, 1982; Lubzens *et al.*, 1985; Ben-Amotz *et al.*, 1987). Only very few authors, Chu and Dupuy (1980) have followed changes in the fatty acid content *during* the different growth phases of culture.

The biochemical composition viz., lipids, fatty acids, proteins and carbohydrates of seven phytoplankton species, *Pavlova lutheri*, Haptophyta-Haptophyceae; *Isochrysis galbana*, Haptophyta-Haptophyceae; *Tetraselmis suecica*, Chlorophyta-Prasinophyceae; *Chaetoceros calcitrans*, Chrysophyta-Bacillariophyceae; *Phaeodactylum tricornutum*, Chrysophyta-Bacillariophyceae, *Rhodomonas* sp., Cryptophyta-Cryptophyceae; and *Heterosigma akashiwo*, Chrysophyta- Raphydophyceae) have been studied by Fernandez-Reiriz *et al.* (1989) at three different phases in the growth curve at the end of one week, two weeks and 80 days, in order to establish for each species the most appropriate growth phase from the point of view of its nutritional and energetic value for bivalve larvae and juveniles.

The use of continuous cultures of marine microalgae to study the nutritional requirements of marine organisms has been suggested in the pioneering works of Scott (1980) and Taub (1980), indicating the potential of these systems to generate microalgal biomass of optimal nutritional quality. It is

important to distinguish between semicontinuous cultures in which dilutions are made every 24 h, being inherently similar to continuous cultures, and extended batch cultures, in which dilutions are made in longer periods and therefore, without the advantage of producing biomass of a stable biochemical composition at a selected growth rate that characterize continuous cultures (Brown *et al.*, 1993).

Otero and Fabregas (1997) have studied the biochemical variability generated through the manipulation of nutrient concentration and renewal rate in semicontinuous cultures of one of the species most commonly used in aquaculture, *Tetraselmis suecica*, as a part of an extensive study on the behavior of several marine microalgal species in semicontinuous culture (Fabregas *et al.*, 1995a, 1996b,c; Otero *et al.*, 1997).

Parsons *et al.* (1961) analyzed chemical composition of eleven species of marine plankton including two members of the Chlorophyceae, *Tetraselmis maculata* and *Dunaliella salina*; two Chrysophyceae, *Monochrysis lutheri* and the Coccolithophore *Syracophaera carterae*; four Bacillariophyceae, *Skeletonema costatum*, *Coscinodiscus* sp., *Chaetoceros* sp. and *Phacodactylum tricornutum*, two Dinophyceae, *Exuviella* sp. and *Amphidinium carteri*; and a representative of the Myxophyceae, *Agmenellum quadruplicatum*. All the species were grown under similar physical and chemical conditions and cells were analyzed during the exponential phase of growth. Chemical analyses consisted of a proximate analysis of each species for ash, protein, carbohydrate and lipid and an analysis for carbon, silicon and phosphorus, as well as quantitative determination of the monosaccharides and aminoacids in hydrolyses of whole cells.

Rodhouse *et al.* (1983), Pillsbury (1985), Ben-Amotz *et al.* (1987) and Whyte (1987) have reported that the Prymnesiophyte *Isochrysis* and the Eustigmatophyte, *Nannochloropsis* are the most commonly used marine unicellular algae in mariculture systems. The marine unicellular algae are rich in C22:6 and C20:5 fatty acids respectively.

Shifrin and Chisholm (1981), Piorreck *et al.* (1984) and Cohen *et al.* (1988) have studied the effect of environmental factors on lipid and fatty acid composition in various eukaryotic algae. Shifrin and Chisholm (1981) and Roessler (1988) have stated that in most algae, enhancement of lipid accumulation is caused by nitrogen-deficient conditions. In diatoms, accumulation of neutral lipids is very much increased under silicon deficiency.

Sukenik and Wahnon (1991) have reported the effects of growth irradiance levels and nitrogen availability on cellular lipid content and fatty acid composition in *Isochrysis* aff. *galbana* (T-ISO). In their study, continuously grown cultures in chemostats and turbidostats have been used to evaluate the effect of environmental parameters on lipid content in *Isochrysis galbana* with special emphasis on fatty acid distribution.

With respect to biochemical composition, the total concentrations of protein, lipid and carbohydrate in microalgae vary substantially with the species and culture conditions (Parsons *et al.*, 1961; Dortch, 1982; Redalje and Laws, 1983; Wikfors *et al.*, 1984; Fabregas *et al.*, 1986; Moal *et al.*, 1987). The levels of specific amino acids, fatty acids, sugars, sterols, vitamins and minerals, within the fractions may be important (Webb and Chu, 1983; Brown *et al.*, 1989).

Volkman *et al.* (1989) have studied the fatty acid and lipid composition of ten species of microalgae used in mariculture. Their study has highlighted the differences in the fatty acid composition of the algal species. In particular, the levels of the polyunsaturated fatty acids (PUFAs) essential for marine animals, i.e., 20:5 ω3 and 22:6 ω3, were deficient in the green algae compared to the brown algae. Microalgae lacking these PUFAs have proved to be inadequate as unialgal (i.e., one species) diets, whereas species with high concentrations of the PUFAs have generally proven to be good to moderate food as unialgal diets (Davis and Guillard, 1958; Walne, 1970; Enright *et al.*, 1986b).

Differences in the polysaccharides of microalgae may be of nutritional significance, since the efficiency with which marine animals digest polysaccharides is dependent on the polysaccharide type (Kristensen, 1972). Parsons *et al.* (1961) have suggested that the nutritional quality of microalgae may be related to proportion of glucose in readily hydrolysable carbohydrate (i.e., oligosaccharides and storage polysaccharides).

Brown (1991) has examined the amino acid and sugar composition of six diatoms, *Chaetoceros calcitrans* (Paulsen) Takano, *Chaetoceros gracilis* Schutt, *Nitzchia closterium* (Ehrenb) W. Smith, *Phaeodactylum tricornutum* Bohlin, *Skeletonema costatum* (Greville) Cleve, *Thalassiosira pseudonana* (Hustedt, clone 3H) Hasle and Heimdal, four prymnesiophytes, *Isochrysis galbana* Parke, *Isochrysis* aff. *galbana* Parke (T-ISO), *Pavlova lutheri* (Droop), Green, *Pavlova salina* (N. Carter) Green, two prasinophytes, *Tetraselmis chui* Butcher, *Tetraselmis suecica* (Kylin) Butcher, two chlorophytes, *Dunaliella tertiolecta* Butcher, *Nannochloris atomus* Butcher, one eustigmatophyte, *Nannochloropsis occulata* (Droop) Green and one Cryptophyte, *Chroomonas salina* (Wislouch) Butcher. The variations in the sugar composition could contribute to differences in the nutritional value of some species, since animals digest polysaccharides of different composition at different rate (Brown, 1991).

Volkman *et al.* (1993) analyzed the gross biochemical composition of Coccoid algae from the Eustigmatophyceae including *Nannochloropsis salina* and an Australian isolate thought to be closely related to *N. oculata* to determine which features of the biochemical composition might prove to be characteristic of this unusual group of microalgae and to assess their potential as feed stocks for mariculture and biotechnology.

Brown *et al.* (1997) have studied the biochemical composition of about 40 species of microalgae belonging to seven algal classes to determine those that may be best adapted to Australian conditions. Microalgae have been found to vary in their proportions of protein (6-52%), carbohydrate (5-23%) and lipid (7-

23%). All species had similar amino acid composition, and are rich in the essential amino acids. Microalgal polysaccharides were variable in sugar composition, but most have high proportions of glucose (21-87%). Diatoms, prymnesiophytes, cryptomonads and eustigmatophytes are rich in one or both of the 20:5 (ω-3) and 22:6 (ω-3) polyunsaturated fatty acids (5-35% total fatty acids), prasinophytes have low to moderate levels of one of the acids (4-10%) whereas chlorophytes are deficient in both acids (0-3%). All the species have relatively high concentrations of ascorbic acid (1-16 mg g^{-1} dry weight) and riboflavin (20-40 μg g^{-1}).

The distribution of total lipid and free and combined fatty acids in natural populations and unialgal cultures of fresh water and marine phytoplankters have been summarized by Strickland (1960, 1965) and Lewin (1962). Ackman *et al.* (1964) have analyzed the fatty acid composition of *Skeletonema costatum* and confirmed the findings of Klenk and Eberhagen (1962a) that the longer chain, highly unsaturated fatty acids characteristic of fish lipid can be produced by some phytoplankton. Parsons and Strickland (1962) have shown that the fat content of eleven species of marine phytoplankton grown in unialgal culture varied from 1.8 to 18% of the dried algal cells. Patterson *et al.* (1994) have studied substantial, qualitative differences in the lipid composition specifically of sterols and long-chain alkenones-derived from six strains of *Isochrysis*. Wikfors and Patterson (1994) have reported growth characteristics, biochemical composition and nutritional value of *Isochrysis* to invertebrates and suggest that a number of strains of this alga differ in terms of their taxonomy, biology and their practical use as mariculture feeds differs as well.

The effect of variation of these parameters on many algal species has been studied in order to better understand their physiology, as well as to answer specific and relevant questions for mass culture and nutrition of bivalves and other herbivores (Uriarte *et al.*, 1993).

Lourenco *et al.* (1997) have compared the biochemical composition of *Tetraselmis gracilis* grown in two different culture media: the Conway medium (Walne, 1966), widely used all over the world, and a commercial medium (Okauchi and Hirano, 1986), which has already been successfully tested in tanks in Brazil (Okauchi, 1985). Results of Lourenco *et al.* (1997) showed that different culture media generate both distinct and variable biochemical profiles in *T. gracilis* with time.

Hayashi *et al.* (1986) elucidated the content and composition of protein fractions in two species of diatoms *Skeletonema costaum* and *Asterionella japonica* and three species of flagellates *Prorocentrum minimum*, *Noctiluca scintillans* and *Olisthodiscus* sp. and their amino acid composition. They have found that the protein of diatoms is nutritionally excellent.

With regard to the tropical isolates, gross chemical and fatty acid compositions of a wide variety of Australian microalgal isolates have been investigated (Renaud *et al.*, 1994).

The sterol profiles of several bivalve molluscs have been studied and these are composed of complex mixtures of C26 to C30 4- desmethyl sterols (Teshima, 1983; Voogt, 1983; Kerr and Baker, 1991).

Knauer *et al.* (1999) have investigated the effect of dietary phytosterols on the sterol composition of spat tissues and found that after a six week feeding period with the microalga, *Chaetoceros muelleri*, *Isochrysis* aff. *galbana* (strain T-ISO) or *Pavlova lutheri*, the sterol profile of spat tissues generally reflected that of the diet, but not all sterols have been assimilated with the same efficiency.

Piveteau *et al.* (1999) have reported that supplying *Skeletonema costatum* to oysters for six weeks improved the condition index, from 26 to 56 as a consequence of an increase in glycogen content from 5.0 to 24.4 g 100 g^{-1} of dry flesh with lipid content remaining steady (9.9 g 100 g^{-1} dry meat). Large changes in fatty acid composition of neutral lipids have been observed. Some fatty acids

of *Skeletonema costatum* such as 16:1ω7 and 20:5ω3, are directly accumulated into lipid fractions. 16: 1ω7 was elongated into 18:1ω7 showing that oysters are able to elongate 16 carbon monounsaturated fatty acids into the corresponding 18 carbon fatty acids. Fatty acids typical of *Skeletonema costatum* (16:4ω1, 16:2ω4, 16:3ω4) are poorly accumulated into natural lipids and phospholipids of oysters suggesting that there is selective accumulation of fatty acids.

Watanabe and Ackman (1972) and Watanabe and Ackman (1974) have estimated the fatty acid composition of *Crassostrea virginica* and *Ostrea edulis,* Bell and Sargent (1985) in *Chlamys islandica,* Beninger and Stephen (1985) in *Tapes decussatus* and *T. philippinarum,* Kluytmans *et al.* (1985) in *Mytilus edulis* and Albentosa *et al.* (1994) in *Venerupis pullastra.* A few studies have been conducted on larval or spat stages by Chu and Webb (1984), Napolitano *et al.* (1988) and Helm *et al.* (1991) on larvae of various oyster spp.; and by Langdon and Waldock (1981) and Albentosa *et al.* (1994) on small size spat.

Trider and Castell (1980) have stated that ω-3 fatty acids are preferentially required over ω-6 fatty acids by *C. virginica.* Langdon and Waldock (1981) have observed that the growth rate of *C. gigas* fed on diets deficient in C20 and C22 highly unsaturated fatty acids can be considerably increased if these fatty acids are additionally supplied within microcapsules. Elongation and desaturation activities of fatty acids are not high enough to sustain optimal growth rates in juvenile oysters (Waldock and Holland, 1984). Thus, these authors have pointed out that highly unsaturated fatty acids, such as 20:5ω-3 or 22:6ω-3, must be supplied in the diet. Elongation and desaturation of linolenic acid to ω-3 PUFA are also highly limited in the *V. pullastra* clam spat, when ω-3 PUFAs were not present in the diet, they were not found in the spat (Albentosa *et al.*, 1994).

Albentosa *et al.* (1996) have studied the fatty acid composition of the *Rudtapes decussatus* spat fed on *Isochrysis galbana*, clone T-ISO, *Tetraselmis suecica* and *Phaeodactylum tricornutum* during four weeks. The fatty acid

composition of the spat has been found to be usually well correlated with that of the diet supplied. Major differences among spat cultures have been found in 14:0, 16:0; 16:1 ω -9, 16:1 ω -7, 18:1 ω -9, 18:2 ω-6, 18:3 ω-3, 18:4 ω -3, 20:5 ω-3, 22:5 ω-6 and 22:6ω-3 fatty acids. These differences have been correlated with the particular fatty acid content of each diet supplied. It has been shown that *R. decussatus* spat have a very low capacity to elongate and desaturate linolenic acid to ω-3 PUFA; so when 20:5ω-3 or 22:6ω-3 were not present in the diet, they were also absent, at least in measurable amounts, in the clams. The absence of any of the essential fatty acids, 20:5ω-3 in T-ISO or 22:6ω-3 in *Tetraselmis*, did not limit spat growth.

In the planktotrophic larval stage and during metamorphosis and early spat growth, neutral lipids (triglycerides) are the major energy reserve of the oyster, *Ostrea edulis* L. (Holland and Spencer, 1973). In contrast, adult oysters of the same speceis have higher levels of glycogen reserves compared to lipid (Russel, 1923, Walne, 1970). Holland and Hannant (1974) have estimated the biochemical composition of three batches of *Ostrea edulis* L. spat during post-settlement growth. For the first few months after settlement the neutral lipid content has been noted to be higher than the glycogen content. However, in three to five-months old young oysters the glycogen level has been high and neutral lipid, level less.

3. MATERIAL AND METHODS

3. 1. SPAT DENSITY

Water Quality for hatchery operation

Raw sea water for maintaining the brood stock and filtered water for hatchery operation were used. Biological filters consisting of sand, gravel, oyster shells were placed *in situ* during the culture operations. Water quality parameters such as pH, temperature, ammonia, dissolved oxygen, phosphate, nitrite, nitrate, silicate and salinity were monitored during the course of the experiments. The parameters did not fluctuate widely during the course of experiments but 10% water exchange was made on alternate days in the culture tanks. Water temperature ranged from 28 to 30°C, pH from 8.1 to 8.3, ammonia from 10 to12 μg at NH_3-N/l, dissolved oxygen from 3 to 4 mg/l, phosphate from 1 to 3 μg at PO_4/l, nitrite from 0.4 to 0.5 μg at N/l, nitrate from 5 to 7 μg at N/l, silicate from 9 to 10 μg at Si/l and salinity from 30 to 34 ppt.

Spawning induction

The pearl oyster *Pinctada fucata* broodstock were cleaned and washed with filtered sea water to remove sediment and fouling organisms. The broodstock were placed in a plastic tray containing filtered sea water and held overnight in an air-conditioned room with ambient water temperature of 22°C. The following morning, the pearl oysters were placed in an aquarium tank of 200 l containing filtered sea water. Spawning was induced by thermal stimulation; the temperature of the aquarium tank sea water was raised to around 30-32°C for 15-30 minutes with Jumo thermometer. When spawning started the pearl oysters were removed from the aquarium tank into another tank and spawning allowed to take place in filtered sea water at 28°C. Fertilized eggs were reared in a 200 l tank. After 24 hours, D-stage veliger larvae were removed from the tank onto a 25μm pore-size mesh screen, counted and kept in larval rearing tanks.

Developmental stages

Feritilisation

Soonafter discharge, the eggs are fertilized with the milt. They assume a spherical shape and the germinal vesicle breaks down. The fertilized egg measures 47.5 μm in diameter. During the process of fertilization the first and second polar bodies are released. The polar bodies persist on the embryo even in the blastula stage.

Cleavage

The first cleavage begins 45min after fertilization resulting in a micromere and a macromere. Now the polar body lies at the cleavage furrow. During the second cleavage the micromere divides into two and the macromere divides unequally into a micromere and a macromere. The stage with three micromere and a macromere is called Trefoil stage. Macromere does not take part in further divisions. Micromeres become smaller and smaller in size after passing through eight cell, sixteen cell and so on and reach morula stage. Then each micromere develops a small cilium, which makes rotation movement of the embryo.

Blastula

The stage is reached 5h after fertilization. Reorientation of cells result in the formation of blastopore and blastocoel.

Gastrula

Gastrulation take place by epiboly. The cells convolute and differentiate into different layers. The archenterons formed at this stage communicates to the exterior through the blastopore. The embryo exhibits phototrophism. It takes 7h to reach the stage.

Trochophore larva

The early trochophore larva develops preoral and postoral tufts of cilia thus marking antero-posterior region of the embryo. A single apical flagellum is developed in the typical trochophore stage. The minute cilia noticed in the blastula stage disappear. A shell gland of the dorsal ectoderm secretes the prodissoconch I. The stage is attained in 10 hours.

Veliger

The veliger stage is reached by the formation of straight hingeline, mantle, rearrangement of preoral cilia into a velum and disappearance of the apical flagellum, preoral and postoral ciliary bands. The straight hinge larva measures on an average 67.5 μm along the antero-posterior and 52.5μm along the dorso ventral axis. The stage is reached in 20 hours.

Umbo Stage

The development of straight-hinge larva to umbo stage is gradual. Typical clam shaped umbo stage is reached between 10-12 days measuring 135 x 130μm. The shell valves are equal and develops mantle folds.

Eye Spot Stage

Eye spot is developed on 15th day when the larva measures 190 x 180 μm. The eye spot is situated at the base of the foot primordium. Eye spot is visible in a spat of 3.9mm. The larva develops ctenidial ridges.

Pediveliger Stage

The foot is developed on 18th day at the size of 200 x 190 μm. The transitional stage from swimming to crawling phase has both velum and foot. Later the foot becomes functional with the disappearance of velum. 2-4 gill filaments are seen at this stage.

Plantigrade

The stage is seen on 20th day and it measures 220 x 200μm. Lapial palps and additional gill filaments develop. The shell growth is by the formation of a very thin, transparent, uniform conchiolin film around the globular shell margin except in the vertex of the umbo region. This is the beginning of the formation of the adult shell or the dissoconch.

Settlement and nursery culture

On day 20, eyed larvae at density of 3/ml were large enough to be retained on a 100 μm pore-size mesh screen were removed from the larval culture tanks and placed in one ton settlement tanks. Black ceramic tiles 20cm x 10cm x 0.5cm in size have been used as spat collectors. Pearl oyster spat settles on them from day 24 over a period of one week. After spat setting in the hatchery they are further grown in nursery tanks. Feeding with mixed algae, especially with *Chaetoceros* enhances the spat growth of 3mm and above with stand transplantation to the new form environment. Water in the settlement tanks was completely exchanged on alternative days and water temperature ranged from 28°C to 32°C during the study. Pearl oyster spat set on the tiles from day 24 to over a period of one week. On day 43, spat were removed from the spat collectors in the settlement tanks.

Optimum spat density

The spat were reared in glass tanks (77.0 x 38.5 x 39.0 cm) of 100 l capacity containing 60 l of seawater with an area of 2964.5 cm^2. Spat used were in the size range of 5-8 mm weighing between 0.030 to 0.050 g. Sea water was changed on alternate days and the spat were fed *ad libitum* twice a day with *Chaetoceros calcitrans*.

Unialgal culture was grown in filtered seawater in test tubes, 100 ml, 200 ml, 2 l conical flasks and 10 l transparent plastic buckets. The water was enriched using Walne's enrichment medium (Walne, 1974). Algal cultures were

maintained at a temperature of 24°C and exposed to a light intensity of 1000 lux for 24 hours.

Spat in the different densities were reared in a volume of 60 l of filtered seawater with three replicates totaling 27 observations for the duration of 56 days. They were maintained at ambient pH (8.10 – 8.30), temperature (28.0 – 32°C) and salinity (30.0 – 34 .0 ppt). Spat densities chosen were 20, 40, 80, and 160 numbers in 60 lit of seawater for each set, which works out to be 67, 135, 270, and 540 individuals/m^2. Spat were removed at the end of the week and shell growth was measured as Dorso Ventral Measurement (DVM) to the nearest 0.1 mm, and weighed (wet weight, WW) to the nearest 0.001 g.

One-way Analysis of Variance (ANOVA) was used to test variations between densities (Snedecor and Cochran, 1967). If the ANOVA gave significant results for a factor, then the specific level of the factor between which differences occurred were determined. Length weight relationship was computed by linear logarithmic equation, $\ln y = a + b \ln x$.

3. 2. ALGAL CONCENTRATION AND NUTRITIVE VALUE OF MICROALGAE

Pearl oyster spat

Pearl oyster spat ranging in initial length from 4 to 6 mm were brought from Chaplakancheru pearl oyster hatchery, Bhogapuram, east coast of India. They were stocked in 100 l glass aquaria tanks (77.0 x 38.5 x 39.0 cm) at a density of 20 spat/60 l. Water in the aquaria was maintained at 28°C-32°C with a salinity of 30-34 ppt and changed every 48 h with the spat being retained on ceramic tiles. Spat were supplied twice daily with 60 cell/µl of algal species, or combination of species. Algal culture growth was monitored to ensure the cultures used were in logarithmic growth phase.

Experiments (with three replicates per treatment) were terminated after 56 days. The length (DorsoVentral Measurement) of 20 spat per aquarium was measured using millimeter scale. For all experiments, the percentage of survival of spat was 100% at the time of termination of the experimental culture.

Algal culture techniques

Pure algal cultures were produced in 200 ml glass flasks, 2 l glass flasks, 2 l plastic jars and 10 l transparent plastic buckets. All species were batch cultured in sea water (salinity 30-34 ppt) using Walne's growth medium (Walne, 1974) at 22-24°C with a 24 h light cycle and were illuminated with cool white fluorescent tube lights at an intensity of 1000 lux at the container side.

Experimental design

Feeding experiments were conducted to determine the species most suited for pearl oyster spat. Feeding was carried out twice a day at interval of 8 hours, at 10 a.m. and 6 p.m. Because, Langton and McKay (1974; 1976) have reported that oyster spat in the laboratory grow faster when fed intermittently than when fed continuously with the same number of algal cells per day. Feeding was completed by 4-5 p.m. and at 6 p.m. microalgae were supplied to the spat. For experiment 1, the five species were fed to spat singly, and in experiment 2, spat were supplied a diet of the algae in combination.

The algae used as food for pearl oyster spat were drawn from 10 l transparent plastic buckets during the exponential phase between days 4 and 6. Since the algal cells are very small and in high concentration, an estimate of the number of algal cells per ml of the culture was made using haemocytometer. The diatoms *Chaetoceros calcitrans* (Paulson) Takano and *Skeletonema costatum* (Greville) Cleve, flagellates *Isochrysis galbana* (Parke), *Nanochloropsis salina* (Hibberd) and *Tetraselmis gracilis* (Kylin) Butcher were tested at concentrations of 15, 30, 60 and 90 cells/μl. Spat density was maintained uniformly at 20 numbers/60 l which was determined as the optimum spat density.

Statistical analysis

Homogeneity of variance was confirmed using the ANOVA test. The data were analyzed with a single factor analysis of variance to examine the effects of species and to determine if there was a significant interaction between the factors. The factors were single and combination of microalgal species. As significant interactions ($P<0.05$) were present in both experiments, the data from all treatment combinations in each of these experiments were then subjected to a single factor analysis of variance.

Nutritive value of single species of microalgae

Five species of micro algae were evaluated for their food value to pearl oyster spat viz. *Chaetoceros calcitrans, Isochrysis galbana, Nanochloropsis salina, Skeletonema costatum* and *Tetraselmis gracilis* singly. All the species were tested at the feeding level of 15 cells/µl, 30 cells/µl, 60 cells/µl and 90 cells/µl concentrations and growth of pearl oyster spat studied using different species and concentrations of the algae.

Spat growth was monitored by measuring spat from each rearing tank along the dorso ventral axis every seventh day. The spat were measured using precision dividers and weighed to 0.001 g accuracy.

To facilitate comparisons between individual treatments, the average spat measurements and the standard error were calculated. Growth measurements were tabulated against the age in days of the spat. Actual growth rate was calculated for eight different periods of spat growth viz. day 1-7,7-14,14-21,21-28,28-35,35-49 and 49-56. Overall growth rate for the period of 8 weeks was calculated. Spat growth was statistically tested for significance after performing the analysis of variance (Snedecor and Cochran, 1967).

Nutritive value of combined species of algae.

The algal species were combined in the following manner and tested for their efficacy.

A) *Chaetoceros calcitrans* + *Isochrysis galbana*

B) *Chaetoceros calcitrans* + *Skeletonema costatum*

C) *Isochrysis galbana* + *Skeletonema costatum*

D) *Chaetoceros calcitrans* + *Isochrysis galbana* + *Nanochloropsis salina*

E) *Chaetoceros calcitrans* + *Isochrysis galbana* + *Skeletonema costatum*

F) *Nanochloropsis salina* + *Skeletonema costatum* + *Tetraselmis gracilis*

G) *Chaetoceros calcitrans* + *Isochrysis galbana* + *Nanochloropsis salina* + *Skeletonema costatum* + *Tetraselmis gracilis*

Spat growth was monitored and measurements made following the same procedure employed for single species of micro algae by measuring a sample of 20 spat from each rearing tank along the dorsoventral axis by using mm scale every seventh day. The spat were then returned to the respective tanks.

To facilitate comparison between individual treatments, the average spat measurements and the standard errors were calculated for each day of sampling. Growth measurements thus calculated were tabulated against the age in days of the spat. Actual growth rate was calculated for eight different periods of spat growth viz., day 1-7, 7-14, 14-21, 21-28, 28-35, 35-42, 42-49 and 49-56. Overall growth rate for day 1 to 56 was also calculated. Growth rate was calculated using the formula i.e. GR = $(\ln LW_t - \ln LW_0)*100/t$ where LW_0 and LW_t are initial and final live weights and t is time in days (Beiras *et al.*, 1993). Spat growth was statistically tested for significance after performing the analysis of variance (Snedecor and Cochran, 1967).

Algal consumption was monitored every 24 hours after feeding. Samples of 2 ml water were removed from the rearing tanks and the algal cell counts taken using a haemocytometer. The counts were taken to represent the level of

algal cells present in the medium at the end of 24 hours from feeding. Algal consumption was calculated by subtracting this from the initial cell concentration.

3.3. BIOCHEMICAL COMPOSITION OF MICROALGAE AND SPAT

Algal culture techniques

Pure algal cultures were produced in 200 ml glass flasks, 2 l glass flasks, 2 l plastic jars and 10 l transparent plastic buckets. All the species were batch cultured in sea water (salinity 30-34 ppt) using Walne's growth medium (Walne, 1974) at 22-24°C with a 24 h light cycle and were illuminated with cool white fluorescent tubes at an intensity of 1000 lux at the container side.

Experimental design

The microalgae used as food for pearl oyster spat were drawn from 10 l transparent plastic buckets during the exponential phase between days 4 and 6. Since the algal cells are very small and in high concentration, an estimate of the number of algal cells per ml of the culture was made using haemocytometer. The diatoms *Chaetoceros calcitrans* (Paulson) Takano (size- 2.5μ) and *Skeletonema costatum* (Greville) Cleve (size- 6μ) and the flagellates *Isochrysis galbana* (Parke) (size- 7-8μm), *Nanochloropsis salina* (Hibberd) (size- 3-4μm x 1.5-1.7μm), and *Tetraselmis gracilis* (Kylin) Butcher (size- 14.8 x 8.6) were tested. The microalgae present in one litre of four days culture were filtered and used for biochemical analysis. For each species the mean value of six estimations for each constituent has been determined.

Statistical analysis

Homogeneity of variance was confirmed using the ANOVA test. The data were analyzed with a single factor analysis of variance to examine the variations between species and to determine if there was a significant interaction between the factors.

Biochemical composition of algae

The five species of microalgae used as diets for the pearl oyster, *Pinctada fucata* spat, namely *Chaetoceros calcitrans*, *Isochrysis galbana*, *Nanochloropsis salina*, *Skeletonema costatum* and *Tetraselmis gracilis* were analyzed for their biochemical composition. The parameters measured were moisture, total carbohydrate, total protein and total lipid. Algae for the analysis were collected during exponential growth phase between days 4 and 6. One litre of algae was centrifuged and the packed cells washed with 0.9% ammonium formate and isotonic sea water, to remove traces of salt (Holland and Gabbot, 1971).

Moisture

The packed cells were weighed on a microbalance (± 0.001 g accuracy) immediately after centrifugation to obtain wet weight. The cells were dried to constant weight at 60^{0} C for 10 to 12 hours in hot air oven, weighed after cooling in a dessicator over silica gel and the percentage of moisture content was calculated.

Total lipid

The gravimetric method of Bligh and Dyer (1959) was used to estimate total lipid. Wet samples of algal cells were homogenized in 10ml of 2:1 (v/v) chloroform: methanol mixture. Extraction was done repeatedly with small volumes of the chloroform-methanol mixture. The lipid extract and washings were transferred to a separating flask and shaken with distilled water. Total lipid was isolated and its weight determined on a microbalance (± 0.001 accuracy) after evaporating solvent and drying the residue over silica gel.

Carbohydrate

Lipid extracted algal samples were used for the estimation of carbohydrate. After lipid extraction with (1: 2 v/v) chloroform-methanol mixture,

the precipitate was air-dried and the carbohydrate extracted in 10% Trichloro acetic Acid (TCA). Aliquots of the supernatant were treated by the method of Dubois *et al.* (1956) using glucose as standard. Optical density readings were taken in a spectrophotometer at 490nm.

Total protein

Total protein was estimated by Lowry's method (Lowry *et al.*, 1951). Fat extracted algal samples were dissolved in 1N sodium hydroxide and the amount of protein determined using Folin-ciocalteu reagent. Readings were calibrated on a spectrophotometer against standard bovine serum albumen at wavelength of 540nm. The level of lipid, carbohydrate and protein in the microalgae was calculated as per cent (%) dry weight.

Biochemical composition of spat

All spat samples (homogenized spat without shell) with maximum Dorso Ventral Measurement (DVM) and weight of 29.15 mm and 1.88 g and minimum DVM and weight of 16 mm and 0.23 g, were washed with 0.9% ammonium formate and isotonic sea water to remove traces of salt. The excess moisture that was present in spat tissue sample was blotted out on filter paper, transferred to an aluminium foil and dried in oven at 60^0 C for 12 hours. The dried spat material was then stored in glass vials in a dessicator until further analysis (Holland and Gabbot, 1971). Spat samples without shell were analyzed for protein, total lipid, phospholipid and total carbohydrate.

Ash

Inorganic matter was estimated gravimetrically after ashing 20mg spat sample in a muffle furnace at 700^0 C for 14 hours. Weights were measured on microbalance (± 0.001g accuracy). For further analysis of protein, total lipid, phospholipid and carbohydrate, 20 mg of pulverized, dried spat material was weighed accurately.

Total lipid

Lipid was extracted using 1:2 (v/v) chloroform: methanol mixture. After keeping at 4^0 C in refrigerator for 10 minutes, the lipid dissolved in the mixture was extracted by centrifugation at 10,000 rpm. Aliquots of the lipid extracts were quantitatively estimated by the method of Marsch and Weinstein (1966) using tripalmitin as standard. After drying aliquots of lipid sample at 37^0 C, sulphuric acid was added and heated for 15 minutes at 200^0 C. On cooling, distilled water was added and the absorbance read in a spectrophotometer at 375nm.

Phospholipid

Using the method described by Holland and Gabbot (1971) lipid phosphorus was estimated as inorganic phosphate after acid digestion using potassium-di-hydrogen phosphate as standard. After drying aliquots of chloroform-extracted lipid at 37^0 C for 15 minutes, samples were digested with phosphorus digestion mixture (50:50 20N H_2SO_4 and 8N perchloric acid) successively at 120^0 C for 1 hour, 200^0 C for 15 minutes and 320^0 C for 2 hour 30 minutes. On cooling ammonium molybdate, aminonaphthosulphonic acid reagent and distilled water were added successively. After heating the reacted mixture, it was cooled and absorbance read in a spectrophotometer at wavelength of 850nm. The values of inorganic phosphorous were raised by a factor of 25 to get value of phospholipid.

Neutral lipid

The values of neutral lipid were calculated by substracting values of phospholipid from total lipid.

Total carbohydrate

A modification of the method of Folin and Malmros (1929) specified by Holland and Gabbot (1971) was used with glucose as a standard. Fat extracted

algal samples were used for analysis of carbohydrate. After fat extraction with chloroform-methanol mixture, the precipitate was air-dried and the carbohydrate extracted in 10% Trichloroacetic Acid (TCA). From the spat homogenate, total carbohydrate was dissolved in cold 15% TCA. Absorbance was read in a spectrophotometer at wavelength of 420nm.

Total protein

The protein precipitated from the 15% Trichloroacetic Acid (TCA) for analysis of carbohydrate was dissolved in warm sodium hydroxide by heating for 30 minutes at 56^0 C. Samples were digested in sulphuric acid and the liberated ammonium nitrogen was determined spectrophotometrically by the phenol hypochlorite method (Solorzano, 1969). Ammonium sulphate was used as standard. The absorbance was determined in spectrophotometer at 535nm. The protein nitrogen values thus obtained were multiplied by 6.25 to get the total protein.

4. RESULTS

4. 1. SPAT DENSITY

Spat density at 20 numbers per 60 l

The mean initial spat DorsoVentral Measurement (DVM) was 8 mm and weight 0.05 g (Table 1). Maximum DVM and weight were 11 mm and 0.128 g and minimum DVM and weight were 6 mm and 0.040 g. On 7th day the average size was 9.5 mm DVM with a weight of 0.12 g (Table 1). Maximum DVM and weight were 13 mm and 0.165 g and minimum DVM and weight were 8 mm and 0.050 g. Growth rate per day in the first week works out to 0.21 mm DVM and weight of 0.01 g (Fig.1 & 2). On 14th day the average size was 12 mm DVM with a weight of 0.175 g (Table1). Maximum DVM and weight were 17 mm and 0.463 g and minimum DVM and weight were 11 mm and 0.128 g. Growth rate per day in the second week was higher than in the first week and worked out to 0.36 mm DVM, and weight of 0.008 g (Fig. 1 & 2). At the end of 3rd week the DVM was 13.8 mm with the weight of 0.386 g. (Table 1). Maximum DVM and weight were 21 mm and 0.887 g and minimum DVM and weight were 13 mm and 0.165 g. The growth rate per day in the third week was calculated to be 0.26 mm in DVM and weight of 0.03 g (Fig.1 & 2). On 28th day the average growth was 16.4 mm DVM with a weight of 0.649 g (Table 1). Maximum DVM and weight were 23 mm and 1.08 g and minimum DVM and weight were 13 mm and 0.165 g. Growth rate per day in the fourth week works out to 0.37 mm DVM and weight of 0.038 g (Fig. 1 & 2).

On 35th day the average growth in DVM was 18 mm with the weight of 0.725 g (Table 1). Maximum DVM and weight were 25 mm and 1.175 g and minimum DVM and weight were 14 mm and 0.19 g. Growth rate per day in the 5th week works out to be 0.23 mm per day in DVM and weight of 0.01 g (Fig.1 & 2). At the end of 6th week the average DVM was 20 mm with a weight of 0.886 g (Table 1). Maximum DVM and weight were 28 mm and 1.777 g and minimum DVM and weight were 15 mm and 0.288 g. The growth rate per day

Table 1. Mean (± SE) growth of *P. fucata* spat at four different densities for 9 weeks

Day	20 / 60 l ($67/m^2$)		40 / 60 l ($134/m^2$)		80 / 60 l ($268/m^2$)		160 / 60 l ($536/m^2$)	
	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Length (mm)	Weight (g)
1	8.0 ± 0.32	0.050± 0.005	8.0± 0.18	0.050± 0.002	8.43± 0.08	0.054± 0.001	7.8± 0.12	0.048± 0.002
7	9.5± 0.35	0.120± 0.008	9.0± 0.18	0.115± 0.004	9.2± 0.09	0.120± 0.001	8.2± 0.15	0.077± 0.003
14	12.0± 0.44	0.175± 0.018	12.5± 0.29	0.185± 0.01	10.8± 0.12	0.158± 0.003	8.7± 0.16	0.101± 0.004
21	13.8± 0.55	0.386± 0.036	13.8± 0.38	0.275± 0.02	11.6± 0.16	0.184± 0.004	9.1± 0.19	0.122± 0.006
28	16.4± 0.68	0.649± 0.062	14.7± 0.41	0.458± 0.025	13.4± 0.17	0.279± 0.007	9.6± 0.2	0.150± 0.007
35	18.0± 0.76	0.725± 0.074	15.6± 0.46	0.557± 0.031	14.5± 0.18	0.391± 0.011	10.0± 0.22	0.151± 0.009
42	20.0± 0.87	0.886± 0.069	16.4± 0.51	0.562± 0.037	15.9± 0.16	0.455± 0.013	10.5± 0.24	0.152± 0.012
49	22.5± 0.98	1.134± 0.105	17.2± 0.53	0.653± 0.043	16.5± 0.2	0.482± 0.017	11.2± 0.25	0.152± 0.015
56	26.75± 0.88	1.242± 0.137	18.0± 0.57	0.724± 0.049	17.2± 0.2	0.506± 0.017	11.9± 0.27	0.153± 0.017

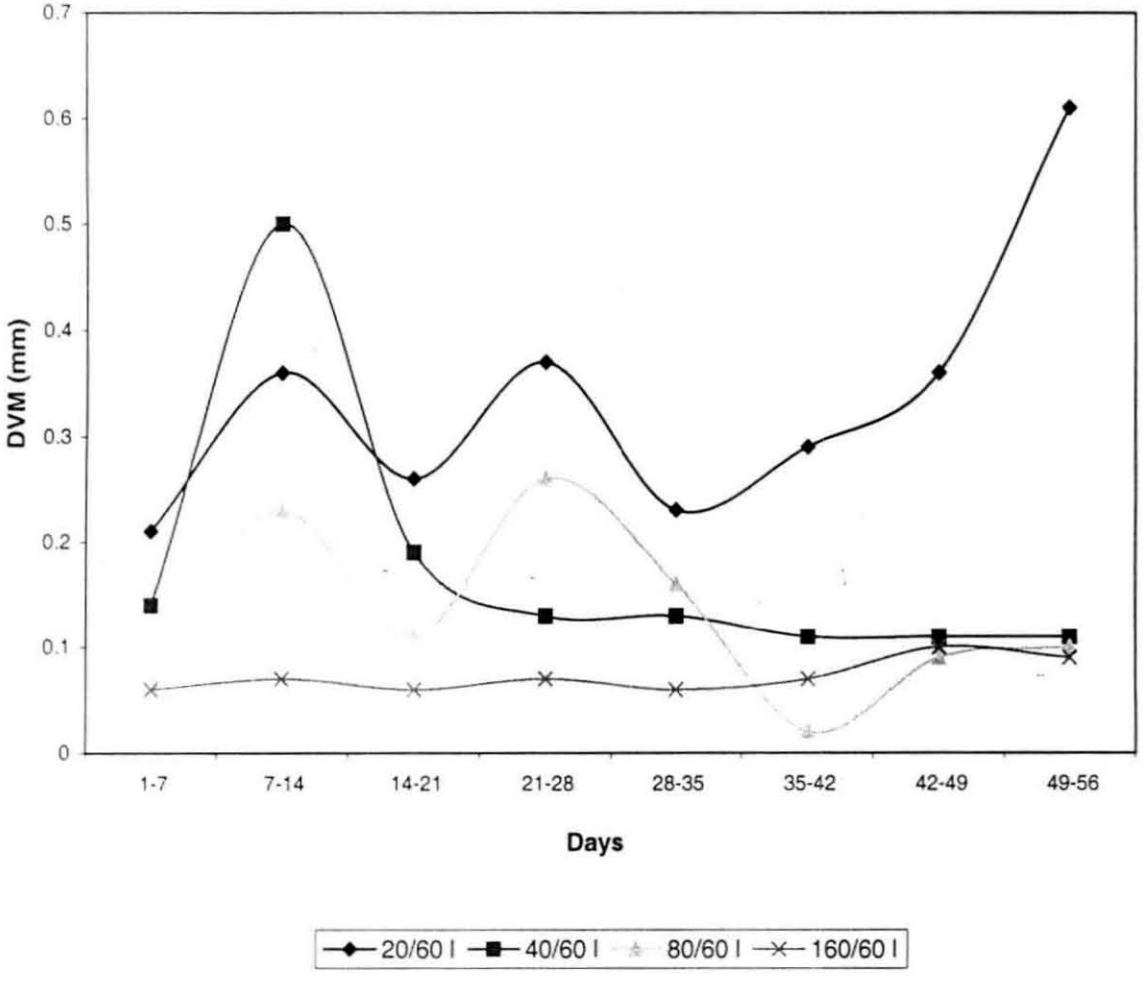

Figure 1. Changes in mean growth rate in DVM (mm) of *P.fucata* spat at different densities

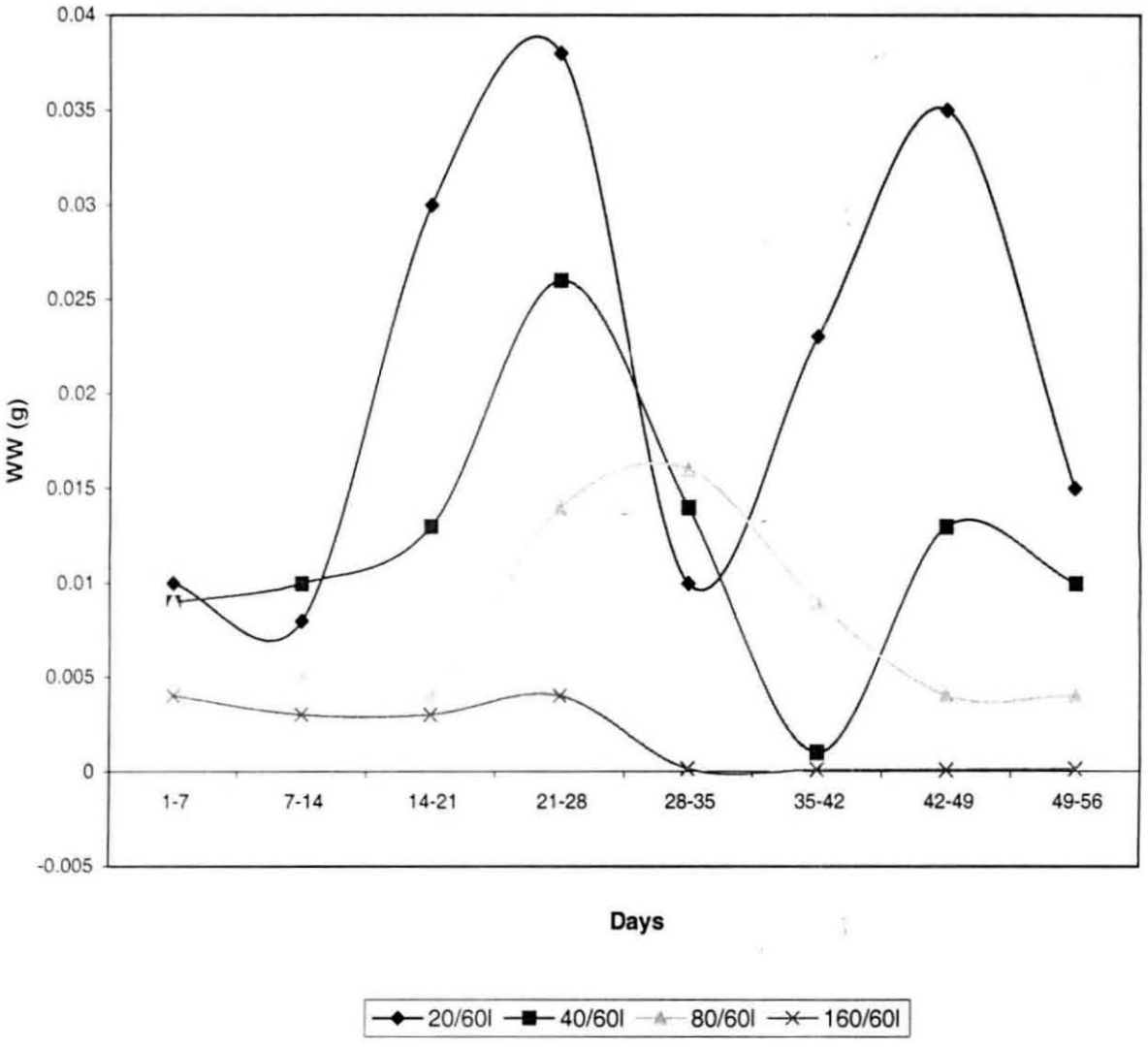

Figure 2. Changes in mean growth rate in wet weight (WW, g) of *P. fucata* spat at different densities

in the sixth week was calculated to be 0.29 mm in DVM and weight of 0.023 g (Fig.1 & 2). On 49th day the average growth in DVM was 22.5 mm with weight of 1.134 g (Table 1). Maximum DVM and weight were 30 mm and 2.135 g and minimum DVM and weight were 16 mm and 0.397g. Growth rate per day in the 7th week works out to be 0.36 mm per day in DVM and weight of 0.035 g (Fig.1 & 2). From the initial spat DVM of 8 mm and weight of 0.05 g spat had grown to 26.75 mm and 1.242 g on 56th day of experiment (Table 1). The maximum DVM and weight were 32 mm and 2.58 g and minimum DVM and weight were 17 mm and 0.463 g. The growth rate per day in the 8th week was calculated to be 0.61 mm in DVM and weight of 0.015 g (Fig.1 & 2).

Spat density at 40 numbers per 60 l

The mean initial spat DorsoVentral Measurement (DVM) was 8 mm and weight was 0.05 g. (Table 1). Maximum DVM and weight were 15 mm and 0.288 g and minimum DVM and weight were 6 mm and 0.035 g. On 7th day the average growth was 9 mm DVM with a weight of 0.115 g (Table 1). Maximum DVM and weight were 17 mm and 0.463 g and minimum DVM and weight were 7 mm and 0.04 g. Growth rate per day in the first week works out to 0.14 mm DVM and weight of 0.009 g (Fig.1 & 2). On 14th day the average growth was 12.5 mm DVM with a weight of 0.185 g (Table 1). Maximum DVM and weight were 21 mm and 0.887 g and minimum DVM and weight were 8 mm and 0.05 g. Growth rate per day in the second week was higher than in the previous week, 0.50 mm DVM and weight of 0.010 g (Fig. 1 & 2). At the end of 3rd week the DVM was 13.8 mm, with the weight of 0.275 g. (Table 1). Maximum DVM and weight were 23 mm and 1.08 g and minimum DVM and weight were 8 mm and 0.05 g. The growth rate per day in the third week was calculated to be 0.19 mm in DVM and weight of 0.013 g (Fig.1 & 2). On 28th day the average growth was 14.7 mm DVM with a weight of 0.458 g. (Table1). Maximum DVM and weight were 25 mm and 1.175g and minimum DVM and weight were 9 mm and 0.06 g. Growth rate per day in the fourth week works out to 0.13 mm DVM and weight of 0.026 g (Fig. 1 & 2).

On 35th day the average growth in DVM was 15.6 mm with weight of 0.557 g (Table1). Maximum DVM and weight were 27 mm and 1.64 g and minimum DVM and weight were 9 mm and 0.060 g. Growth rate per day in the 5th week works out to 0.13 mm per day in DVM and weight of 0.014 g (Fig.1 & 2). At the end of 6th week the DVM was 16.4 mm and with weight of 0.562 g. (Table1). Maximum DVM and weight were 28 mm and 1.777 g and minimum DVM and weight were 9 mm and 0.06 g. The growth rate per day in the sixth week was calculated to be 0.11 mm in DVM and weight of 0.001 g (Fig.1 & 2). On 49th day the average growth in DVM was 17.2 mm with weight of 0.653 g (Table1). Maximum DVM and weight were 29 mm and 1.913 g and minimum DVM and weight were 10 mm and 0.08 g. Growth rate per day in the 7th week works out to be 0.11 mm per day in DVM and weight of 0.013 g (Fig.1 & 2). From the initial spat DVM of 8 mm and weight of 0.05 g spat had grown to 18 mm and 0.724 g on 56th day of experiment (Table1). Maximum DVM and weight were 30 mm and 2.135 g and minimum DVM and weight were 11 mm and 0.128 g. The growth rate per day in the 8th week was calculated to be 0.11 mm in DVM and weight of 0.010 g (Fig.1 & 2).

Spat density at 80 numbers per 60 l

The mean initial spat DorsoVentral Measurement (DVM) was 8.43 mm and weight 0.054 g (Table 1). Maximum DVM and weight were 13 mm and 0.165 g and minimum DVM and weight were 7 mm and 0.04 g. On 7th day the average growth was 9.2 mm DVM with a weight of 0.12 g. (Table 1). Maximum DVM and weight were 18 mm and 0.562 g and minimum DVM and weight were 8 mm and 0.05 g. Growth rate per day in the first week works out to 0.11 mm DVM and weight of 0.009 g (Fig.1 & 2). On 14th day the average growth was 10.8 mm DVM with a weight of 0.158 g (Table 1). Maximum DVM and weight were 19 mm and 0.66 g and minimum DVM and weight were 9 mm and 0.06 g. Growth rate per day in the second week works out to 0.23 mm DVM and weight of 0.005 g (Fig. 1 & 2). At the end of 3rd week the DVM was 11.6 mm, with weight of 0.184 g. (Table 1). Maximum DVM and weight were 20 mm and 0.731 g and minimum

DVM and weight were 9 mm and 0.06 g. The growth rate per day in the third week was calculated to be 0.11 mm in DVM and weight of 0.004 g (Fig.1 & 2). On 28th day the average growth was 13.4 mm DVM with a weight of 0.279 g (Table 1). Maximum DVM and weight were 21 mm and 0.887 g and minimum DVM and weight were 9 mm and 0.06 g. Growth rate per day in the fourth week works out to 0.26 mm DVM and weight of 0.014 g (Fig. 1 & 2).

On 35th day the average growth in DVM was 14.5 mm with the weight of 0.391g (Table 1). Maximum DVM and weight were 23 mm and 1.08 g and minimum DVM and weight were 10 mm and 0.08 g. Growth rate per day in the 5th week works out to be 0.16 mm per day in DVM and weight of 0.016 g (Fig.1 & 2). At the end of 6th week the DVM was 15.9 mm and weight of 0.455 g (Table 1). Maximum DVM and weight were 24 mm and 1.14g and minimum DVM and weight were 10 mm and 0.08g. The growth rate per day in the sixth week was calculated to be 0.20 mm in DVM and weight of 0.009g (Fig.1 & 2). On 49th day the average growth in DVM was 16.5 mm with the weight of 0.482g (Table1). Maximum DVM and weight were 25 mm and 1.175g and minimum DVM and weight were 10 mm and 0.08g. Growth rate per day in the 7th week works out to be 0.09 mm per day in DVM and weight of 0.004g (Fig.1 & 2). From the initial spat DVM of 8.43 mm and weight of 0.054g spat had grown to 17.19 mm and 0.506g on 56th day of experiment (Table1). Maximum DVM and weight were 26 mm and 1.21g and minimum DVM and weight were 11 mm and 0.128g. The growth rate per day in the 8th week was calculated to be 0.10 mm in DVM and weight of 0.004g (Fig.1 & 2).

Spat density at 160 numbers per 60 l

The mean initial spat DorsoVentral Measurement (DVM) was 7.8 mm and weight was 0.048g (Table 1). Maximum DVM and weight were 15 mm and 0.288g and minimum DVM and weight were 5 mm and 0.03g. On 7th day the average growth was 8.2 mm DVM with a weight of 0.077g (Table 1). Maximum DVM and weight were 16 mm and 0.397g and minimum DVM and weight were

5 mm and 0.03g. Growth rate per day in the first week works out to 0.06 mm DVM and weight of 0.004g (Fig.1 & 2). On 14th day the average growth was 8.7 mm DVM with a weight of 0.101g (Table1). Maximum DVM and weight were 17 mm and 0.463g and minimum DVM and weight were 6 mm and 0.035g. Growth rate per day in the second week works out to 0.07 mm DVM and weight of 0.003g (Fig. 1 & 2). At the end of 3rd week the DVM was 9.1 mm, with the weight of 0.122g (Table 1). Maximum DVM and weight were 18 mm and 0.562g and minimum DVM and weight were 6 mm and 0.035g. The growth rate per day in the third week was calculated to be 0.06 mm in DVM and weight of 0.003g (Fig.1 & 2). On 28th day the average growth was 9.6 mm DVM with a weight of 0.150g (Table1). Maximum DVM and weight were 18 mm and 0.562g and minimum DVM and weight were 7 mm and 0.04g. Growth rate per day in the fourth week works out to 0.07 mm DVM and weight of 0.004g (Fig. 1 & 2).

On 35th day the average growth in DVM was 10 mm with weight of 0.151g (Table1). Maximum DVM and weight were 19 mm and 0.660g and minimum DVM and weight were 7 mm and 0.04g. Growth rate per day in the 5th week works out to be 0.06 mm per day in DVM and weight of 0.00014g (Fig.1 & 2). At the end of 6th week the DVM was 10.5 mm and with the weight of 0.152g (Table1). Maximum DVM and weight were 19 mm and 0.66g and minimum DVM and weight were 7 mm and 0.04g. The growth rate per day in the sixth week was calculated to be 0.07 mm in DVM and weight of 0.00007g (Fig.1 & 2). On 49th day the average growth in DVM was 11.2 mm with the weight of 0.152g (Table1). Maximum DVM and weight were 20 mm and 0.731g and minimum DVM and weight were 8 mm and 0.05g. Growth rate per day in the 7th week works out to be 0.10 mm per day in DVM and weight of 0.00007g (Fig.1 & 2). From the initial spat DVM of 7.8 mm and weight of 0.048g spat had grown to 11.86 mm and 0.153g on 56th day of experiment (Table1). Maximum DVM and weight were 21 mm and 0.887g and minimum DVM and weight were 8 mm and 0.05g. The growth rate per day in the 8th week was calculated to be 0.09 mm in DVM and weight of 0.00013g (Fig.1 & 2).

Mean and standard error of various densities are given in Table 1. Growth of spat at density of 20 spat per 60 l was higher than those at higher densities. Spat density of 20 and 40 spat per 60 l consistently showed higher values than other densities. A decreasing trend is observed in the case of 80 spat and 160 spat per 60 l of seawater while an increasing trend is noticed for 20 and 40 spat per 60 l.

One-way ANOVA (Table 2) indicated significant variations between treatments ($P<0.01$). Comparison of densities indicated that there is no variation between closely placed densities (Table 3). Length-weight relationship calculated from transformed data show significant deviation of **b** from 3.0 with high r^2-values (Table 4). If b value is less than 3.0 the relationship will be non significant and the b value is more than 3.0 the relationship will be significant.

Table 2. A one-way ANOVA between *P. fucata* spat densities for DVM (mm) and wet weight (WW, g)

A. DVM (mm)					
Source of variation	SS	df	MS	F	P Value
Treatment	205.37	3	68.46	4.38	P<0.01
Error	499.95	32	15.62		
B. Wet weight (WW, g)					
Source of variation	SS	df	MS	F	P Value
Treatment	1.06	3	0.35	4.94	P<0.01
Error	2.29	32	0.07		

Table 3. **Density comparisons between treatments for both DVM and WW based on ANOVA tables**

Density	40/60l	80/60l	160/60l
20/60l	n.s	sig	sig
40/60l		n.s	sig
80/60l			n.s

n.s - not significant

sig - significant

Table 4. **Morphometric relationships for *P. fucata* spat following log transformation of values for DVM (mm) and WW (g) (n = 27)**

Density	Regression equation	r^2
20/60 l	lnWW = 2.6394 lnDVM – 8.3456	0.94
40/60 l	lnWW = 2.8332 lnDVM – 8.9282	0.97
80/60 l	lnWW = 3.3230 lnDVM – 10.1298	0.94
160/60 l	lnWW = 1.7602 lnDVM – 6.3029	0.68

4. 2. ALGAL CONCENTRATION AND NUTRITIVE VALUE OF MICROALGAE

Single diet experiments

Chaetoceros calcitrans

Growth of pearl oyster spat fed with *Chaetoceros calcitrans* indicated slow growth at a concentration of 15 cells/μl and maximum growth at 60 cells/μl (Table 5). The growth, however, was not significantly different ($P>0.05$) at different concentrations (Table 6). Length-weight relationship showed isometric growth with **b** values ranging from 2.19 to 2.31 (Table 16). Growth rates DVM and WW (Fig. 3 and 4) showed differences with peak growth during third week for DVM while for WW it was during the fourth week. Length of spat showed steep increase in the first two weeks and declined to original rates by the fourth week. From the fourth week, rates were low and indicated declining trends till the end of experiment.

Isochrysis galbana

A similar pattern of growth as that of *C. calcitrans* was observed with minimum at 15 cells/μl and maximum at 60 cells/μl concentrations respectively (Table 7) with non-significant differences (Table 8). Growth rate in DVM (Fig. 5), however, showed differences at the end of first and second weeks before attaining a uniform growth at the third week. The peak was at the fourth week with declining trend thereafter. In terms of WW, the growth rate at all concentrations indicated a close uniform pattern with peak at fourth week (Fig. 6). Length-weight relationships showed lower values of **b** with range from 2.06 to 2.18 (Table 16).

Table 5. **Growth of pearl oyster spat at different concentrations of *Chaetoceros calcitrans* (Spat density: 20/60 l)**

Week	Mean size of spat							
	15 Cells / µl		30 Cells / µl		60 Cells / µl		90 Cells / µl	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	5.50 ± 0.17	0.032 ± 0.001	5.75 ± 0.26	0.034 ± 0.001	6.00 ± 0.24	0.035 ± 0.001	5.75 ± 0.26	0.034 ± 0.001
2	6.00 ± 0.26	0.034 ± 0.001	6.85 ± 0.30	0.360 ± 0.002	7.30 ± 0.21	0.038 ± 0.001	6.71 ± 0.30	0.036 ± 0.002
3	7.90 ± 0.28	0.063 ± 0.003	8.95 ± 0.32	0.066 ± 0.006	9.50 ± 0.41	0.067 ± 0.009	8.78 ± 0.48	0.065 ± 0.009
4	13.40 ± 0.37	0.122 ± 0.022	14.20 ± 0.38	0.123 ± 0.028	15.80 ± 0.49	0.125 ± 0.041	14.47 ± 0.77	0.123 ± 0.046
5	15.20 ± 0.40	0.336 ± 0.036	16.20 ± 0.43	0.339 ± 0.039	17.60 ± 0.53	0.340 ± 0.050	16.33 ± 0.85	0.338 ± 0.060
6	17.00 ± 0.45	0.468 ± 0.039	18.80 ± 0.55	0.472 ± 0.056	19.40 ± 0.60	0.474 ± 0.060	18.40 ± 0.94	0.471 ± 0.078
7	18.70 ± 0.52	0.548 ± 0.053	20.80 ± 0.67	0.550 ± 0.056	21.30 ± 0.74	0.568 ± 0.050	20.26 ± 1.07	0.550 ± 0.070
8	19.50 ± 0.58	0.603 ± 0.050	22.80 ± 0.65	0.606 ± 0.068	23.90 ± 0.83	0.635 ± 0.070	22.06 ± 1.27	0.606 ± 0.140
9	21.00 ± 0.68	0.619 ± 0.050	24.50 ± 0.66	0.625 ± 2.190	26.35 ± 0.74	0.650 ± 1.960	23.95 ± 1.34	0.618 ± 0.170

Table 6. A one-way ANOVA between *Chaetoceros calcitrans* cell concentrations for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	SS	df	MS	F	P
Treatment	90.03	3	30.01	0.71	>0.01
Error	4406.18	104	42.37		
B. Wet weight (WW, g)					
Source of variation	SS	df	MS	F	P
Treatment	0.005	3	0.002	0.03	>0.01
Error	6.204	104	0.060		

Table 7. **Growth of pearl oyster spat at different concentrations of *Isochrysis galbana* (spat density: 20 / 60 l)**

Week	Mean size of spat							
	15 Cells / µl		30 Cells / µl		60 Cells / µl		90 Cells / µl	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	4.10 ± 0.06	0.028 ± 0.0003	5.15 ± 0.16	0.030 ± 0.001	6.00 ± 0.24	0.031 ± 0.001	4.75 ± 0.21	0.030 ± 0.001
2	4.90 ± 0.12	0.029 ± 0.001	5.25 ± 0.20	0.031 ± 0.001	6.40 ± 0.33	0.033 ± 0.002	5.51 ± 0.27	0.031 ± 0.001
3	5.85 ± 0.13	0.035 ± 0.001	6.20 ± 0.22	0.038 ± 0.001	6.90 ± 0.27	0.039 ± 0.002	6.31 ± 0.30	0.037 ± 0.002
4	6.80 ± 0.13	0.065 ± 0.001	7.05 ± 0.27	0.067 ± 0.002	8.00 ± 0.31	0.070 ± 0.005	7.28 ± 0.32	0.067 ± 0.002
5	9.86 ± 0.27	0.151 ± 0.006	10.50 ± 0.53	0.153 ± 0.010	11.00 ± 0.58	0.156 ± 0.024	10.36 ± 0.46	0.153 ± 0.010
6	12.00 ± 0.29	0.228 ± 0.010	13.50 ± 0.70	0.232 ± 0.040	14.20 ± 0.84	0.235 ± 0.063	13.23 ± 0.63	0.232 ± 0.030
7	15.60 ± 0.47	0.354 ± 0.030	16.50 ± 0.92	0.357 ± 1.130	17.30 ± 1.10	0.358 ± 0.080	16.46 ± 0.96	0.358 ± 0.080
8	17.80 ± 0.57	0.502 ± 0.050	20.50 ± 1.23	0.506 ± 0.130	21.70 ± 1.15	0.508 ± 0.150	20.00 ± 1.05	0.505 ± 1.360
9	19.30 ± 0.70	0.567 ± 0.060	22.50 ± 1.20	0.570 ± 0.140	23.60 ± 1.09	0.600 ± 0.150	21.80 ± 1.24	0.579 ± 1.350

Table 8. A one-way ANOVA between *Isochrysis galbana* cell concentrations for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	60.02	3	20.01	0.52	>0.01
Error	3981	104	38.29		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.001	3	0.00	0.01	>0.01
Error	4.348	104	0.042		

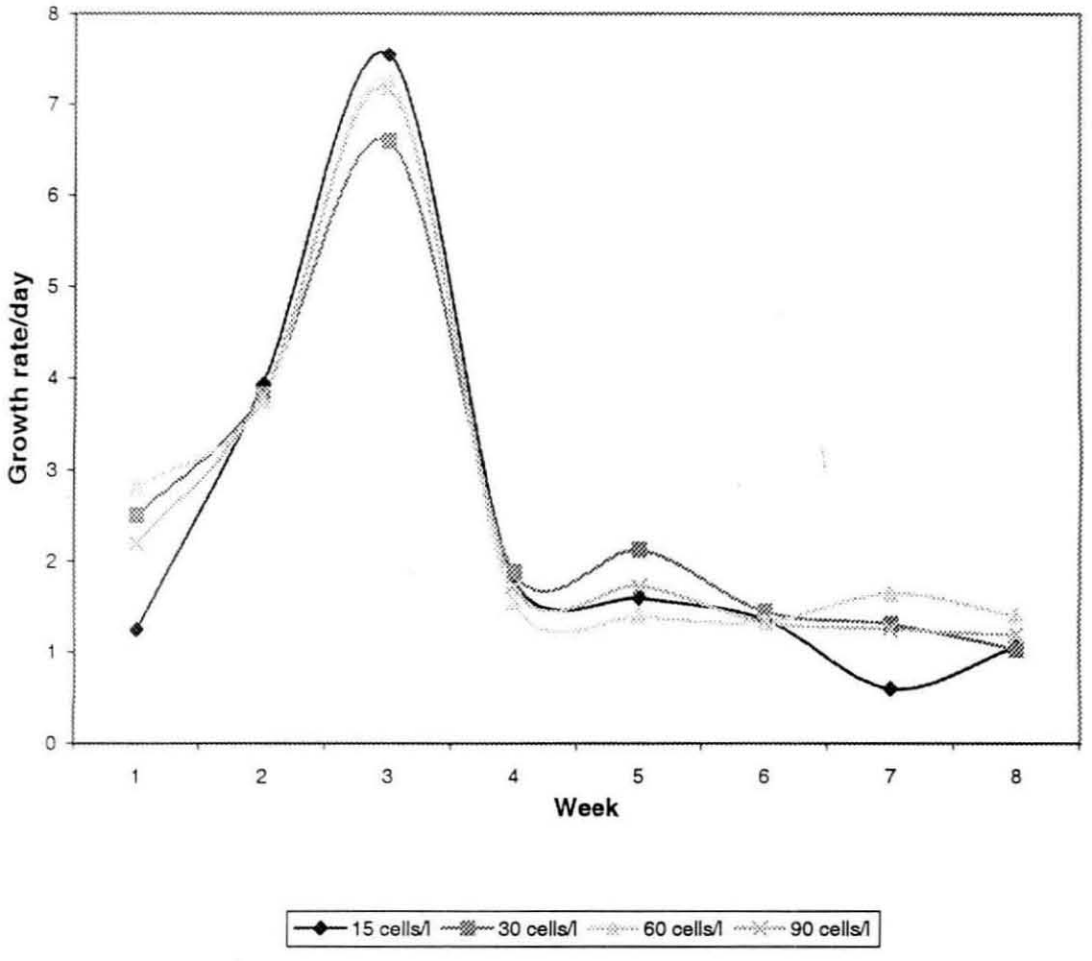

Figure 3. Growth rate in DVM (mm) of pearl oyster spat *P. fucata* fed at different concentrations of *Chaetoceros calcitrans*

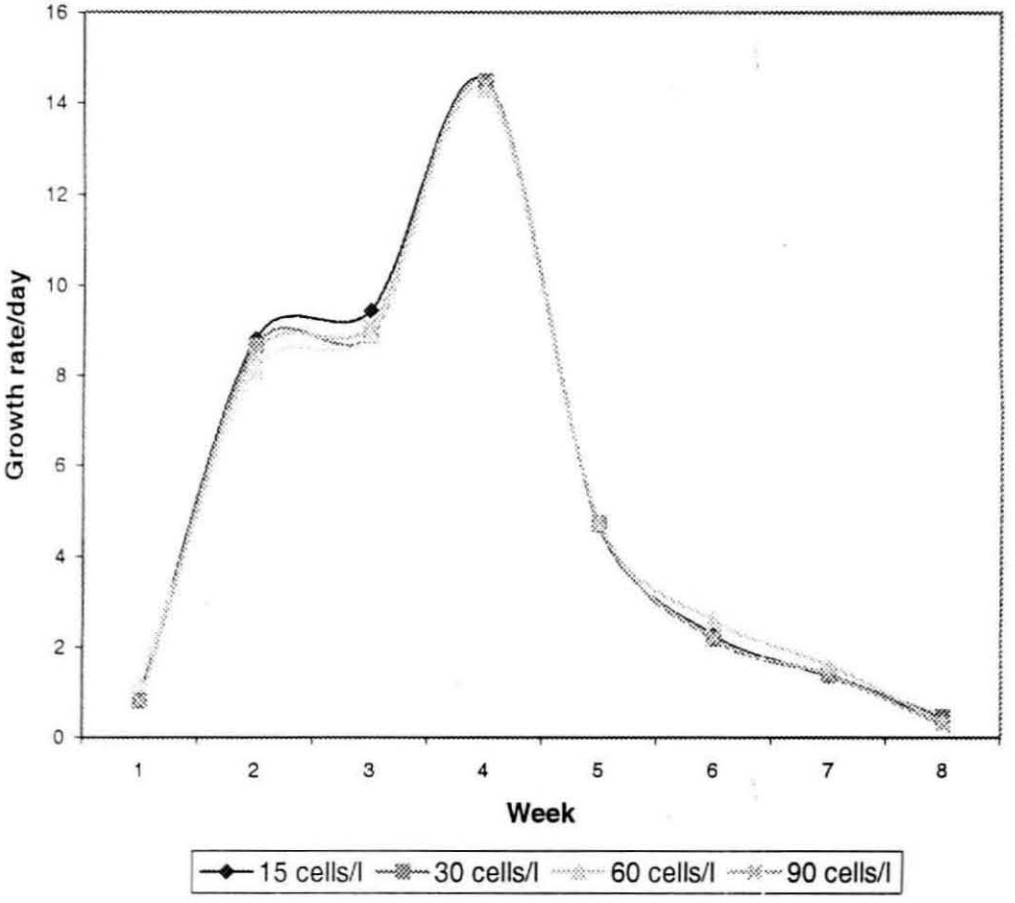

Figure 4. Growth rate of *P. fucata* spat in wet weight (ww, g) when fed at different concentrations of *Chaetoceros calcitrans*

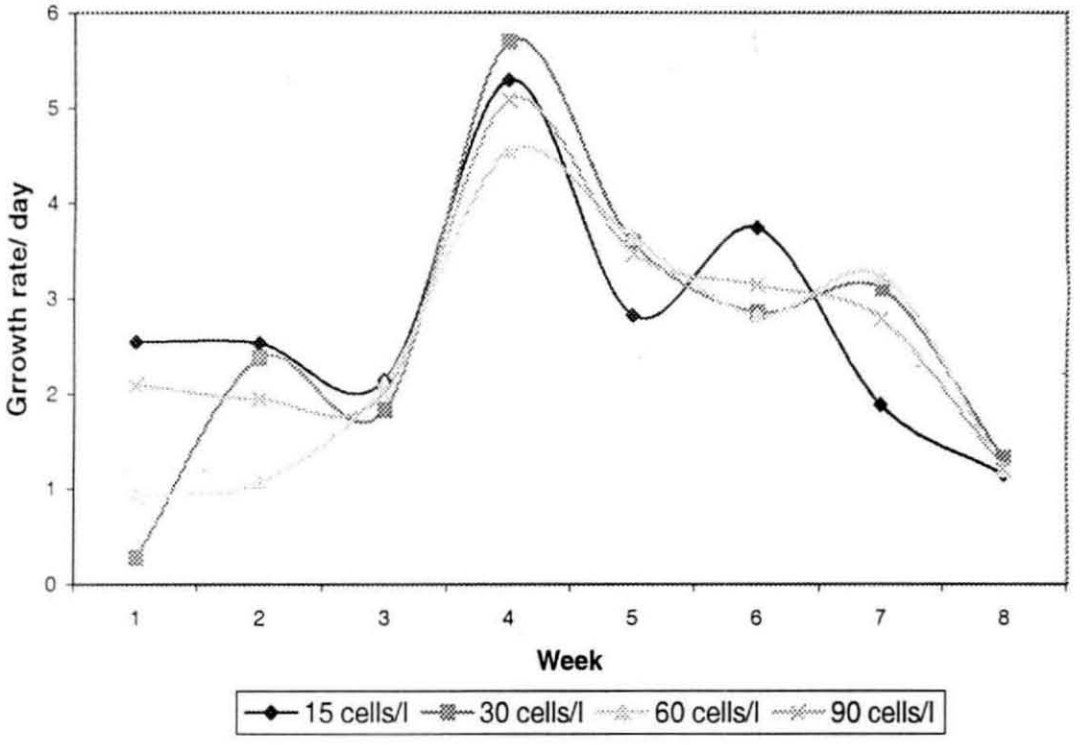

Figure 5. Growth rate in DVM (mm) of *P. fucata* spat fed at different concentrations of *Isochrysis galbana*

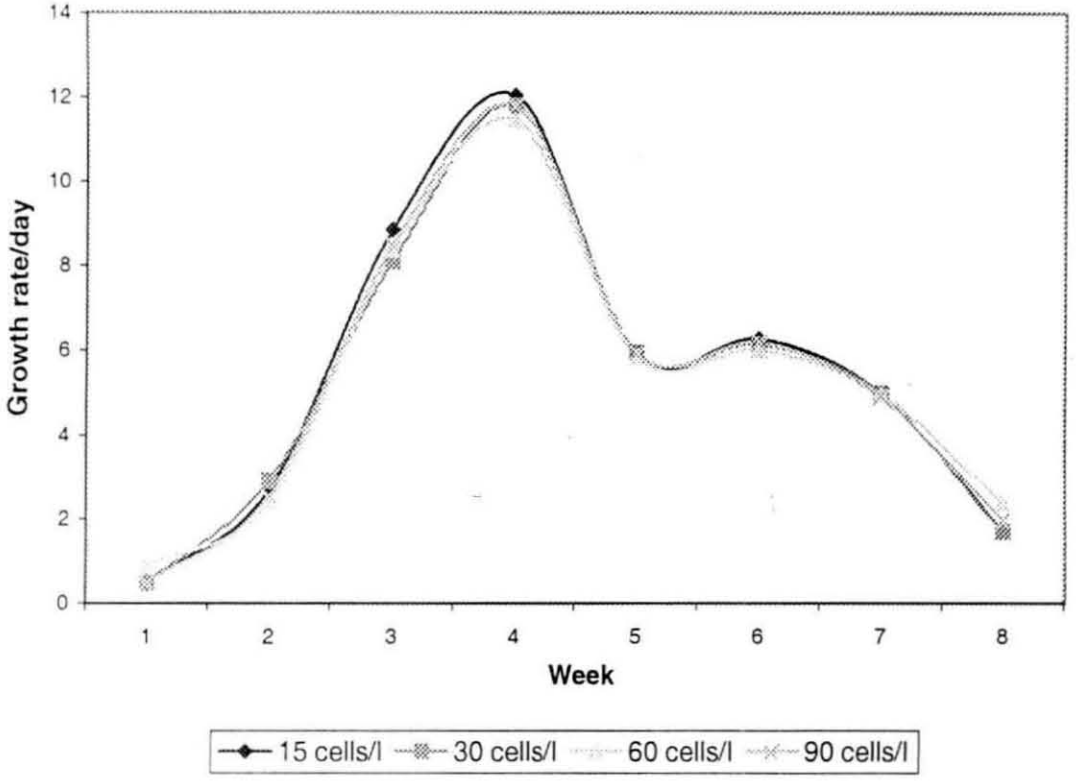

Figure 6. Gowth rate of *P. fucata* spat in wet weight (WW, g) when fed at different concentrations of *Isochrysis galbana*

Nanochloropsis salina

As in the case of *C. calcitrans* and *I. galbana*, the minimum and maximum growth were at 15 and 60 cells/μl (Table 9). The concentration of cells showed ($P<0.01$) differences in DVM (Table 10) mainly due to the higher growth at 30 and 60 cells/μl. Comparison of treatments indicated significant differences between 15 and 30 cells/μl and 15 and 60 cells/μl and between 60 and 90 cells/μl (Table 11). There was no significant differences ($P>0.05$) when compared with WW (Table 10). Linear regression between length and weight showed wide ranges from 1.99 to 2.69 (Table 16). Growth rates for DVM indicated alternating high and low values with a general declining trend (Fig. 7). In terms of WW, the growth rate was uniform for different cell concentrations peaking early in the second week, and stabilizing in third and fourth weeks before declining to low rates by the sixth week of experiment (Fig. 8).

Skeletonema costatum

The general pattern of minimum growth at 15 cells/μl and maximum at 60 cells/μl was also observed in the case of *S. costatum* (Table 12) with non-significant differences between concentrations (Table 13). The **b** values of the length-weight relationship ranged from 2.15 to 2.64 (Table 16). The growth rate in DVM peaked early in the second week (Fig. 9) and showed fluctuating growth rates as the experiment progressed. A single prominent peak was observed in growth rates for WW in the fourth week after a progressive increase (Fig. 10).

Tetraselmis gracilis

The general patterns of low growth at lower concentration (15 cells/μl) and optimum growth at 60 cells/μl was repeated in the case of *T. gracilis* (Table 14) with non significant ($P>0.05$) values between treatments (Table 15). Wide variation in growth rates of DVM was observed (Fig. 11) with peaks during second, fourth and seventh weeks at 60 cells/μl. Growth rates for WW

Table 9. **Growth of pearl oyster spat at different concentrations of *Nanochloropsis salina* (spat density: 20 / 60 l)**

Week	Mean size of spat							
	15 Cells / µl		30 Cells / µl		60 Cells / µl		90 Cells / µl	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	4.50 ± 0.11	0.030 ± 0.001	5.00 ± 0.14	0.033 ± 0.001	5.00 ± 0.21	0.035 ± 0.001	5.00 ± 0.14	0.033 ± 0.001
2	5.00 ± 0.22	0.032 ± 0.001	5.50 ± 0.23	0.034 ± 0.001	5.70 ± 0.30	0.035 ± 0.001	5.50 ± 0.21	0.033 ± 0.001
3	6.00 ± 0.22	0.055 ± 0.001	7.00 ± 0.38	0.057 ± 0.003	8.00 ± 0.58	0.059 ± 0.009	6.50 ± 0.21	0.057 ± 0.001
4	6.50 ± 0.34	0.090 ± 0.002	8.50 ± 0.58	0.092 ± 0.010	9.80 ± 0.79	0.095 ± 0.010	7.00 ± 0.30	0.092 ± 0.002
5	7.50 ± 0.34	0.151 ± 0.002	10.00 ± 0.71	0.154 ± 0.020	12.50 ± 1.05	0.157 ± 0.050	8.00 ± 0.30	0.153 ± 0.003
6	9.00 ± 0.46	0.208 ± 0.010	12.00 ± 0.90	0.211 ± 0.040	14.30 ± 1.19	0.213 ± 0.070	10.00 ± 0.49	0.210 ± 0.010
7	10.00 ± 0.46	0.262 ± 0.010	13.50 ± 0.96	0.263 ± 0.060	16.80 ± 1.51	0.267 ± 0.100	11.00 ± 0.49	0.264 ± 0.010
8	11.50 ± 0.57	0.413 ± 0.010	15.00 ± 1.01	0.417 ± 0.080	17.50 ± 1.59	0.419 ± 0.110	12.50 ± 0.62	0.416 ± 0.030
9	13.00 ± 0.69	0.508 ± 0.030	16.00 ± 1.01	0.518 ± 0.080	18.00 ± 1.70	0.524 ± 0.130	14.00 ± 0.72	0.517 ± 0.040

Table 10. A one-way ANOVA between *Nanochloropsis salina* cell concentrations for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	233.85	3	77.95	5.38	<0.01
Error	1506.68	104	14.48		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.001	3	0.00	0.01	>0.01
Error	2.894	104	0.028		

Table 11. **Algal cell concentration comparisons between treatments for DVM based on ANOVA tables**

Algal cell concentration	**30 cells/µl**	**60 cells/µl**	**90 cells/µl**
15 cells/µl	sig	sig	n.s.
30 cells/µl		n.s.	n.s.
60 cells/µl			sig

n.s.- not significant

sig - significant

Table 12. **Growth of pearl oyster spat at different concentrations of *Skeletonema costatum* (spat density: 20 / 60 l)**

Week	Mean size of spat							
	15 Cells / µl		30 Cells / µl		60 Cells / µl		90 Cells / µl	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	4.00 ± 0.00	0.027 ± 0.001	4.00 ± 0.00	0.029 ± 0.001	4.40 ± 0.11	0.030 ± 0.010	4.20 ± 0.09	0.029 ± 0.001
2	4.50 ± 0.11	0.030 ± 0.001	4.70 ± 0.10	0.031 ± 0.001	5.00 ± 0.19	0.032 ± 0.001	4.50 ± 0.13	0.031 ± 0.001
3	6.00 ± 0.16	0.034 ± 0.001	7.00 ± 0.17	0.036 ± 0.001	7.80 ± 0.38	0.038 ± 0.004	6.60 ± 0.28	0.036 ± 0.001
4	7.00 ± 0.16	0.044 ± 0.001	8.00 ± 0.17	0.045 ± 0.001	8.60 ± 0.43	0.046 ± 0.010	7.50 ± 0.32	0.045 ± 0.002
5	7.80 ± 0.20	0.112 ± 0.001	9.40 ± 0.27	0.115 ± 0.010	10.50 ± 0.71	0.117 ± 0.020	8.00 ± 0.39	0.115 ± 0.004
6	8.90 ± 0.23	0.168 ± 0.005	10.00 ± 0.36	0.170 ± 0.010	11.20 ± 0.78	0.173 ± 0.030	9.40 ± 0.49	0.170 ± 0.010
7	10.00 ± 0.29	0.252 ± 0.007	11.50 ± 0.47	0.254 ± 0.010	13.80 ± 0.83	0.257 ± 0.050	10.80 ± 0.62	0.255 ± 0.020
8	11.60 ± 0.37	0.346 ± 0.010	13.00 ± 0.56	0.347 ± 0.030	15.00 ± 0.90	0.350 ± 0.070	12.00 ± 0.69	0.347 ± 0.030
9	13.00 ± 0.47	0.435 ± 0.020	14.80 ± 0.66	0.436 ± 0.040	16.13 ± 0.94	0.440 ± 0.080	13.50 ± 0.78	0.437 ± 0.050

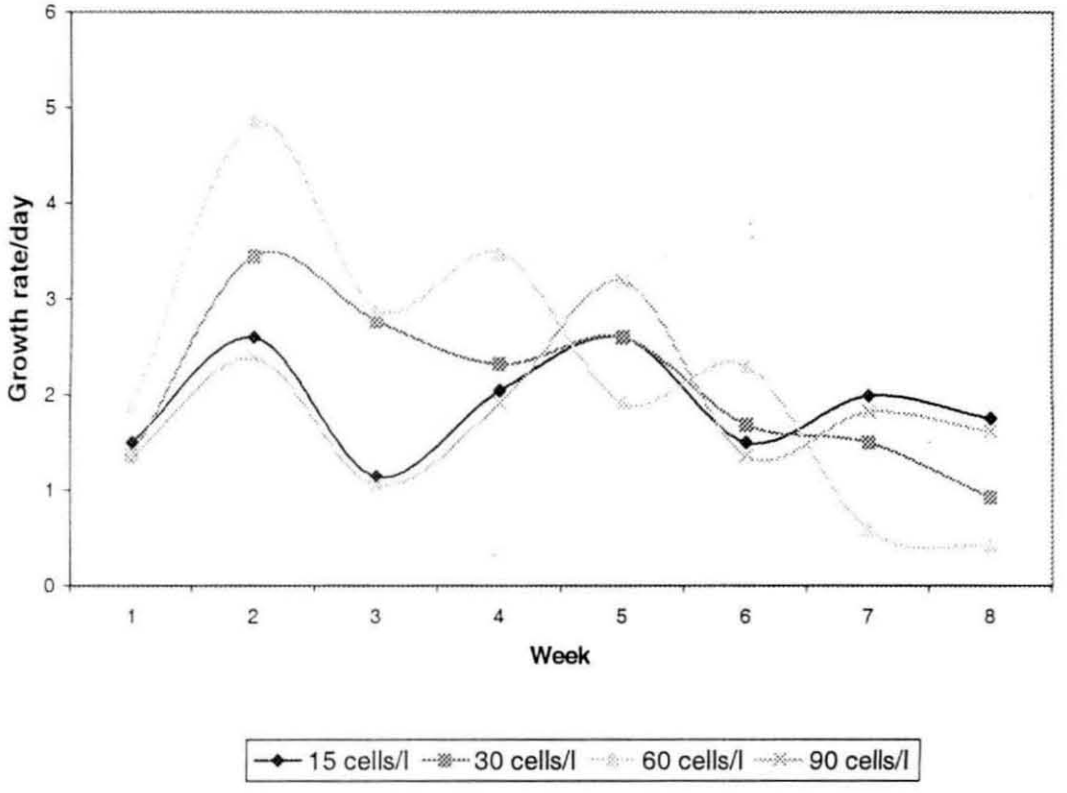

Figure 7. Growth rate of *P. fucata* in DVM (mm) when fed at different concentrations of *Nanochloropsis salina*

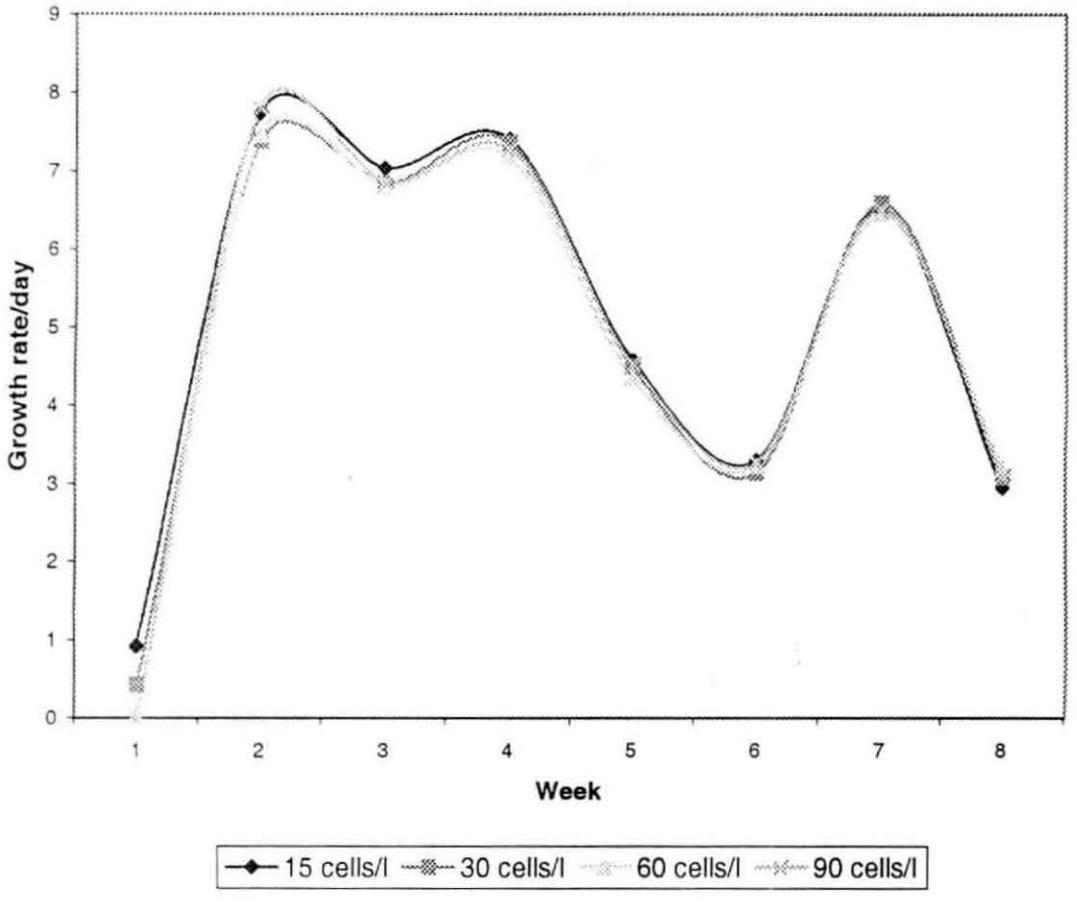

Figure 8. Growth rate of *P. fucata* spat in wet weight (WW, g) when fed at different concentrations of *Nanochloropsis salina*

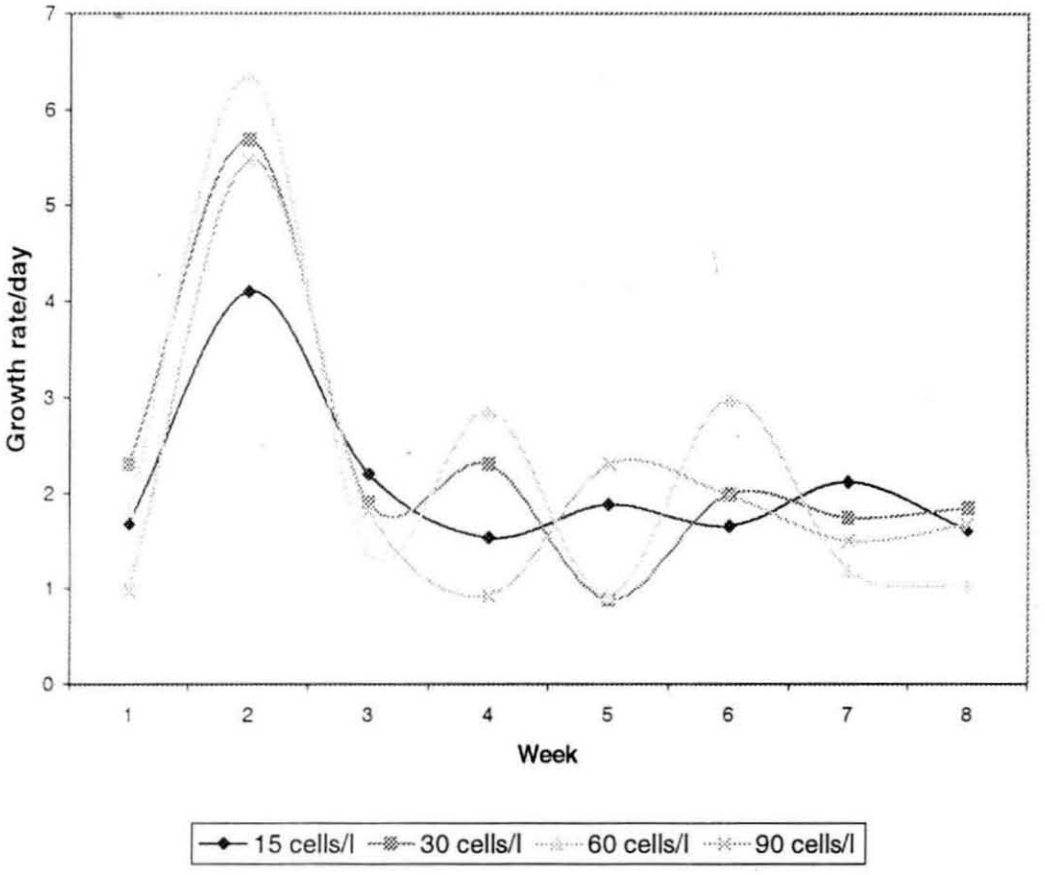

Figure 9. Growth rate of *P. fucata* spat in DVM (mm) when fed at different concentrations of *Skeletonema costatum*

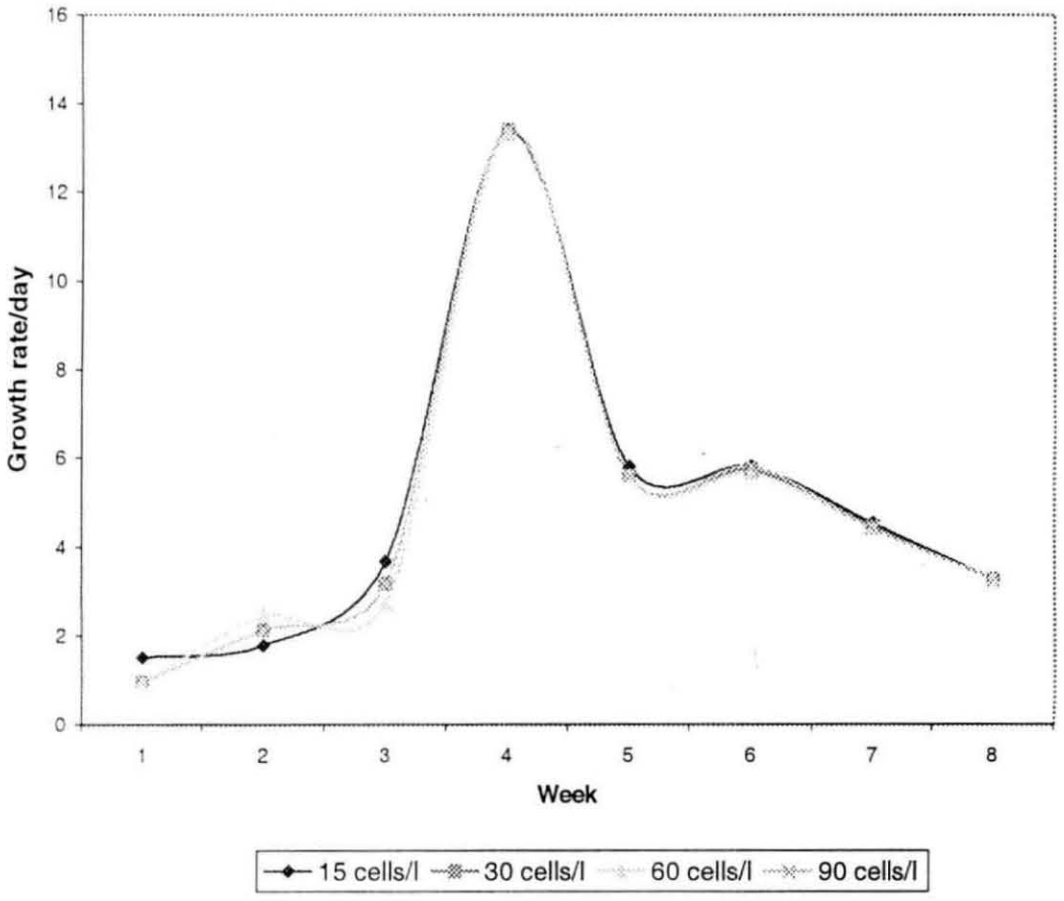

Figure 10. Growth rate of *P. fucata* spat in wet weight (WW, g) when fed at different concentrations of *Skeletonema costatum*

peaked by fourth week and declined in the fifth week before peaking again in the seventh week (Fig. 12). Regression values showed lower weight gain with increase in length with **b** values ranging from 2.15 to 2.73 (Table 16).

Comparison of growth between microalgae

The percentage increase in growth for the 5 species of algae at different concentrations is presented in Table 13. Except for *I. galbana* and *S. costatum* maximum increase in growth is observed at 60 cells/µl. *I. galbana* showed maximum increase at 15 cells/µl while *S. costatum* showed maximum increase at 30 cells/µl *I. galbana* at 15 cells/µl recorded a growth increase of 370% while the lowest value of 167% was observed for *T. gracilis* at 15 cells/µl. A one-way ANOVA between the various microalgae for DVM at 60 cells/µl showed significant ($P<0.01$) variation between treatments (Table 18). This significance was due to the higher growth in spat fed on a diet of *C. calcitrans*.

Combined algal diets

Two species diets

Among the two species diets, maximum growth was recorded for the mixture *C. calcitrans* and *S. costatum* (Table 20). The pattern of growth during the experiment was nearly uniform with early peaking for the combination of *I. galbana* and *S. costaum*. The three groups showed declining growth by sixth to seventh week before improving towards the end of experiment (Fig. 13 & 14). The percentage increase in growth was also maximum for the mixture of *C. calcitrans* and *I. galbana* followed by the combination of *I. galbana* and *S. costatum* (Table 23). Linear regression analysis, however, did not give high values of **b** for this combination. The **b** value was only 1.93 while the other two combinations had values were above 2.0 (Table 22).

Table 13. A one-way ANOVA between *Skeletonema costautm* cell concentrations for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	73.36	3	24.46	2.04	>0.01
Error	1244.08	104	11.96		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.00	3	0.00	0.00	>0.01
Error	2.23	104	0.02		

Table 14. **Growth of pearl oyster spat at different concentrations of *Tetraselmis gracilis* (spat density: 20 / 60 l)**

Week	Mean size of spat							
	15 Cells / µl		30 Cells / µl		60 Cells / µl		90 Cells / µl	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	4.50 ± 0.17	0.030 ± 0.001	4.70 ± 0.16	0.032 ± 0.001	4.90 ± 0.12	0.033 ± 0.001	4.50 ± 0.11	0.032 ± 0.001
2	5.00 ± 0.22	0.032 ± 0.001	5.00 ± 0.25	0.033 ± 0.001	5.20 ± 0.18	0.035 ± 0.001	5.00 ± 0.22	0.033 ± 0.001
3	6.40 ± 0.25	0.051 ± 0.001	7.00 ± 0.29	0.054 ± 0.003	7.30 ± 0.24	0.056 ± 0.002	7.00 ± 0.36	0.054 ± 0.002
4	7.50 ± 0.28	0.087 ± 0.002	8.00 ± 0.29	0.089 ± 0.005	8.40 ± 0.31	0.091 ± 0.007	8.00 ± 0.36	0.089 ± 0.004
5	8.00 ± 0.38	0.150 ± 0.005	9.40 ± 0.38	0.153 ± 0.009	10.80 ± 0.45	0.154 ±0.010	8.50 ± 0.47	0.152 ± 0.008
6	9.00 ± 0.38	0.181 ± 0.008	10.00 ± 0.47	0.184 ± 0.010	11.10 ± 0.52	0.185 ±0.020	9.80 ± 0.50	0.183 ± 0.010
7	9.80 ± 0.43	0.218 ± 0.010	10.60 ± 0.46	0.221 ± 0.010	11.70 ± 0.58	0.224 ±0.020	10.20 ± 0.59	0.221 ± 0.010
8	10.50 ± 0.52	0.322 ± 0.010	12.00 ± 0.56	0.326 ± 0.020	15.00 ± 0.76	0.328 ±0.060	11.00 ± 0.64	0.325 ± 0.010
9	12.00 ± 0.62	0.377 ± 0.020	13.5 ± 0.65	0.386 ± 0.040	16.00 ± 0.76	0.388 ± 0.060	12.70 ± 0.77	0.384 ± 0.030

Table 15. A one-way ANOVA between *Tetraselmis gracilis* cell concentrations for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	57.91	3	19.30	2.12	>0.01
Error	949.16	104	9.127		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.00	3	0.00	0.01	>0.01
Error	1.565	104	0.015		

Table 16. Morphometric relationships for *P. fucata* spat following log transformation of values for Dorso Ventral Margin (DVM) and wet weight (WW, g) (n = 27).

Concentration	Regression equation	r^2
Chaetoceros calcitrans		
15 cells/µl	lnWW = 2.31 lnDVM – 7.51	0.95
30 cells/µl	lnWW = 2.24 lnDVM – 7.53	0.96
60 cells/µl	lnWW = 2.19 lnDVM – 7.51	0.94
90 cells/µl	lnWW = 2.25 lnDVM – 7.55	0.95
Isochrysis galbana		
15 cells/µl	lnWW = 2.10 lnDVM – 6.78	0.98
30 cells/µl	lnWW = 2.06 lnDVM – 6.88	0.98
60 cells/µl	lnWW = 2.18 lnDVM – 7.31	0.97
90 cells/µl	lnWW = 2.10 lnDVM – 6.95	0.98
Nanochloropsis salina		
15 cells/µl	lnWW = 2.69 lnDVM – 7.53	0.93
30 cells/µl	lnWW = 2.28 lnDVM – 7.17	0.95
60 cells/µl	lnWW = 1.99 lnDVM – 6.77	0.94
90 cells/µl	lnWW = 2.62 lnDVM – 7.59	0.92
Skeletonema costatum		
15 cells/µl	lnWW = 2.64 lnDVM – 7.69	0.89
30 cells/µl	lnWW = 2.32 lnDVM – 7.26	0.86
60 cells/µl	lnWW = 2.15 lnDVM – 7.09	0.86
90 cells/µl	lnWW = 2.48 lnDVM – 7.42	0.87
Tetraselmis gracilis		
15 cells/µl	lnWW = 2.73 lnDVM – 7.76	0.92
30 cells/µl	lnWW = 2.37 lnDVM – 7.20	0.92
60 cells/µl	lnWW = 2.15 lnDVM – 6.94	0.98
90 cells/µl	lnWW = 2.47 lnDVM – 7.30	0.90

Table 17. The growth of spat of *Pinctada fucata* in a 56 day period when fed at different rations by cell density of microalgae

Diet	Cells (μl^{-1})	Initial shell length (mm)	Final shell length (mm)	% Growth increase
C. calcitrans	15	5.50 ± 0.17	21.00 ± 0.68	281.81
	30	5.75 ± 0.24	24.50 ± 0.66	326.08
	60	6.00 ± 0.24	26.35 ± 0.74	339.16
	90	5.75 ± 0.26	23.95 ± 1.34	316.52
I. galbana	15	4.10 ± 0.06	19.30 ± 0.70	370.73
	30	5.15 ± 0.16	22.50 ± 1.20	336.89
	60	6.00 ± 0.24	23.60 ± 1.09	293.33
	90	4.75 ± 0.21	21.80 ± 1.24	358.94
N. salina	15	4.50 ± 0.11	13.00 ± 0.69	188.88
	30	5.00 ± 0.14	16.00 ± 1.01	220.00
	60	5.00 ± 0.21	18.00 ± 1.70	260.00
	90	5.00 ± 0.14	14.00 ± 0.72	180.00
S. costatum	15	4.00 ± 0.00	13.00 ± 0.47	225.00
	30	4.00 ± 0.00	14.80 ± 0.66	270.00
	60	4.40 ± 0.11	16.13 ± 0.94	266.59
	90	4.20 ± 0.09	13.50 ± 0.78	221.42
T. gracilis	15	4.50 ± 0.17	12.00 ± 0.62	166.66
	30	4.70 ± 0.16	13.50 ± 0.65	187.23
	60	4.90 ± 0.12	16.00 ± 0.76	226.53
	90	4.50 ± 0.11	12.70 ± 0.77	182.22

Table 18. A one-way ANOVA between various single species of microalgae for DVM (mm) and wet weight (WW, g) of *P. fucata* at 60 cells/µl

A. DVM (mm)					
Source of variation	SS	df	MS	F	P
Treatment	701.10	4	175.28	5.97	<0.01
Error	3818.07	130	29.37		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.47	4	0.12	3.42	>0.01
Error	4.49	130	0.04		

Table 19. Single species of microalgal comparisons between treatments for DVM based on ANOVA tables

Microalgae	*I. galbana*	*N. salina*	*S. costatum*	*T. gracilis*
I. galbana	sig	sig	sig	sig
N. salina		n.s.	n.s.	n.s.
S. costatum			n.s.	n.s.
T. gracilis				n.s.

n.s.- not significant

sig - significant

Table 20. **Growth of pearl oyster spat at different combinations of microalgae (spat density: 20 / 60 l)**

Week	Cc + Ig		Cc + Sc		Ig + Sc	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	4.00 ± 0.00	0.034 ± 0.001	5.75 ± 0.09	0.038 ± 0.001	5.00 ± 0.10	0.033 ± 0.001
2	4.60 ± 0.11	0.042 ± 0.001	7.00 ± 0.16	0.040 ± 0.001	5.80 ± 0.17	0.034 ± 0.001
3	5.70 ± 0.14	0.066 ± 0.001	8.30 ± 0.25	0.082 ± 0.003	7.60 ± 0.25	0.070 ± 0.001
4	7.80 ± 0.20	0.231 ± 0.002	10.50 ± 0.32	0.162 ± 0.009	10.00 ± 0.42	0.138 ± 0.009
5	10.70 ± 0.34	0.350 ± 0.010	13.20 ± 0.38	0.284 ± 0.020	12.70 ± 0.55	0.243 ± 0.010
6	13.40 ± 0.69	0.517 ± 0.040	16.00 ± 0.46	0.368 ± 0.030	14.30 ± 0.64	0.416 ± 0.030
7	15.40 ± 0.71	0.688 ± 0.060	19.00 ± 0.46	0.476 ± 0.040	16.00 ± 0.73	0.503 ± 0.040
8	20.30 ± 0.87	0.891 ± 0.080	20.70 ± 0.64	0.685 ± 0.050	18.00 ± 0.73	0.611 ± 0.050
9	25.50 ± 0.83	1.020 ± 0.120	27.00 ± 0.71	0.998 ± 0.130	24.60 ± 0.82	0.620 ± 0.100

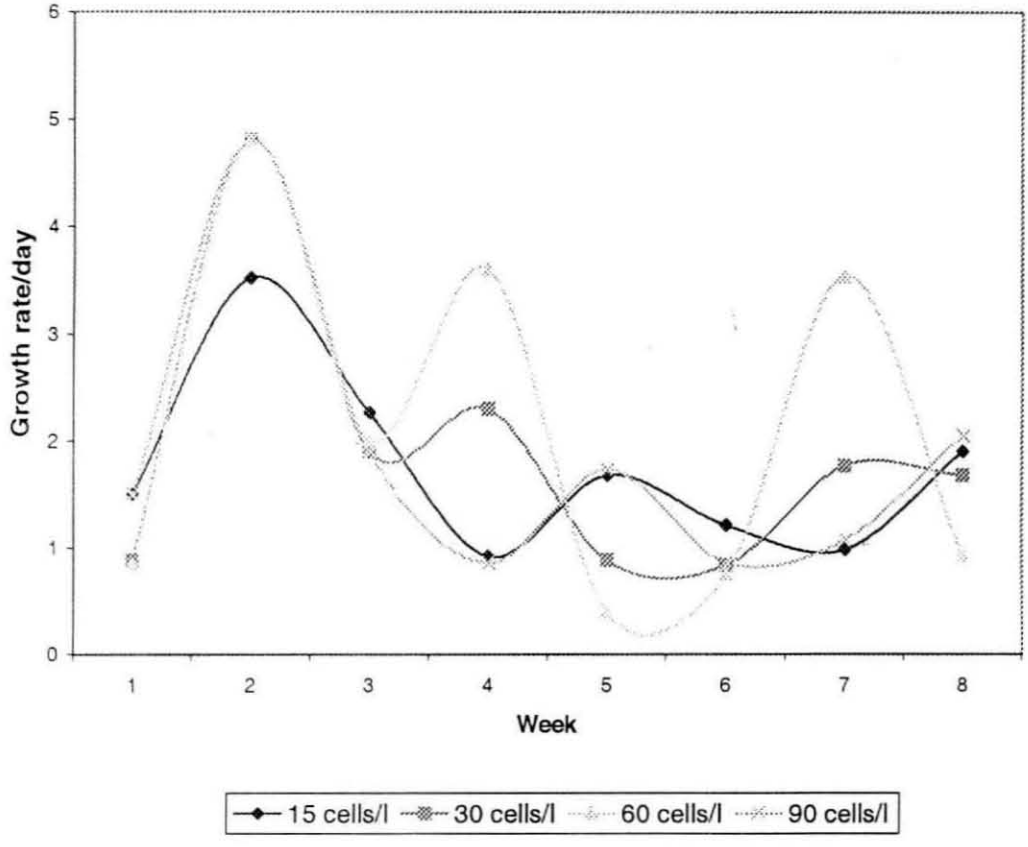

Figure 11. Growth rate of *P. fucata* in DVM (mm) when fed at different concentrations of *Tetraselmis gracilis*

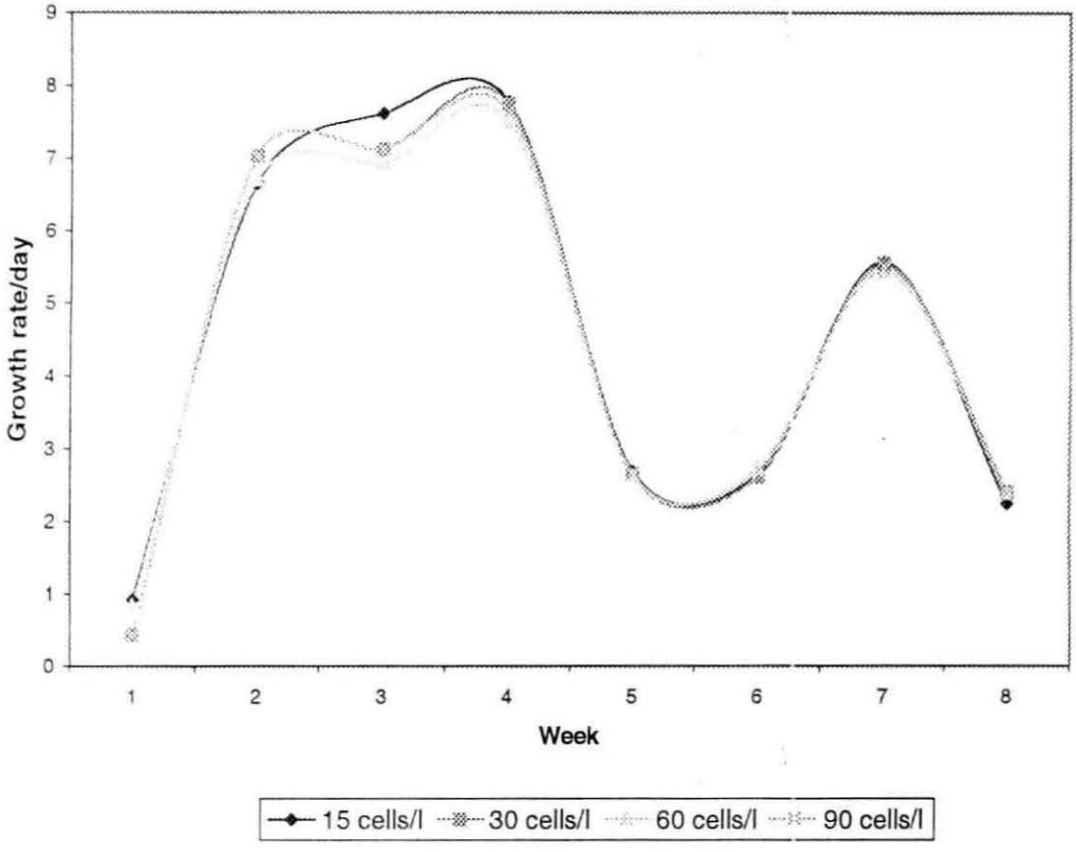

Figure 12. Growth rate of *P. fucata* in wet weight (WW, g) when fed at different concentrations of *Tetraselmis gracilis*

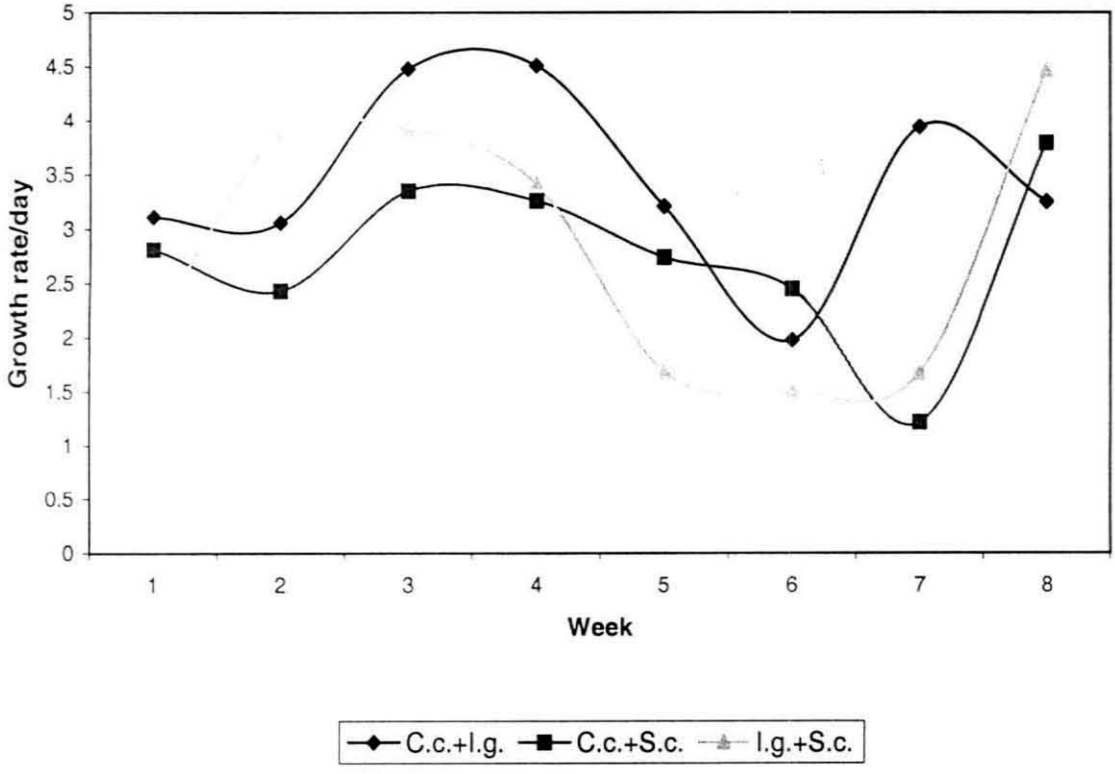

Figure 13. Growth rate of *P.fucata* spat in Dorso Ventral Measurement (DVM, mm) when fed with different combinations of microalgae

Abbreviations: C.c.-*C. calcitrans*, I.s.- *I.galbana*, S.c- *S. costatum*

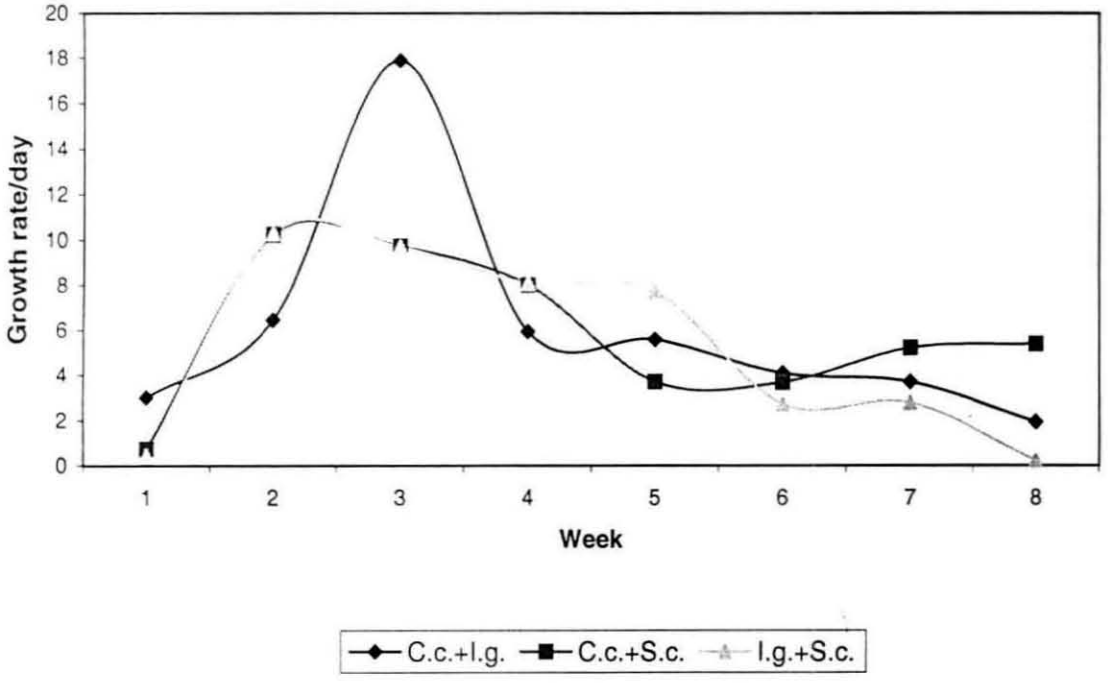

Figure 14. Growth rate of *P. fucata* spat in wet weight (WW, g) when fed with different combinations of microalgae

Abbreviations: C.c.-*C. calcitrans*, I. g.- *I. galbana*, N.s.-*N. salina*, S.c.- *S.costatum*,T.g.-T.gracilis

Three species diets

The mixture of *C. calcitrans*, *I. galbana* and *N. salina* indicated better growth of spat closely followed by the second combination of *C. calcitrans*, *I. galbana* and *S. costatum* (Table 21). The mixture of *N. salina*, *S. costatum* and *T. gracilis* had the least growth when compared with either three or two species diets. The first and third combination (*C. calcitrans* + *I. galbana* + *N. salina* and *N. salina* + *S. costatum* + *T. gracilis*) showed a uniform growth pattern of spat with growth rate of 6mm/day by the fourth week (Fig. 15 & 16). The second mixture (*C. calcitrans* + *I. galbana* + *S. costatum*) peaked early in the third week after an initial decline and indicated a decreasing trend by the fourth week. Percentage increase in growth was similar for the first and second combination but was very low for the third mixture without *C. calcitrans* (Table 23). Regression analysis of DVM on WW indicated high value for the second diet (Table 22).

Five species diet

Growth of spat in terms of DVM and WW was the highest when fed on a diet comprising all the 5 species of microalgae (Table 21). Pattern of growth showed a peak by third week for DVM after an initial decline and decreased to a low rate of 1mm/day by the sixth week (Fig. 17). In terms of WW, the growth rate was progressive till third week and spurted to a rate of 18mm/day by fourth week and declined to a rate of 2mm/day by the sixth week. The percentage increase in growth was comparable to the first and second combination three species diets (Table 23). The length-weight relation was the best among all the combination with **b** value of 2.56 (Table 22).

Table 21. **Growth of pearl oyster *P. fucata* spat at different combinations of microalgae (spat density: 20 / 60 l)**

Week	Mean size of spat							
	Cc + Ig + Ns + Sc + Tg		Cc + Ig + Ns		Cc + Ig + Sc		Ns + Sc + Tg	
	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)	DVM (mm)	Weight (g)
1	5.35 ± 0.19	0.028 ± 0.001	5.30 ± 0.17	0.033 ± 0.001	5.15 ± 0.20	0.031 ± 0.001	5.25 ± 0.19	0.031 ± 0.001
2	7.80 ± 0.43	0.045 ± 0.007	7.00 ± 0.24	0.058 ± 0.001	7.60 ± 0.32	0.042 ± 0.003	5.80 ± 0.27	0.032 ± 0.001
3	10.00 ± 0.56	0.091 ± 0.010	9.60 ± 0.40	0.096 ± 0.010	9.20 ± 0.49	0.079 ± 0.010	7.50 ± 0.41	0.035 ± 0.004
4	15.20 ± 0.69	0.188 ± 0.050	12.40 ± 0.37	0.335 ± 0.010	15.80 ± 0.58	0.175 ± 0.040	9.00 ± 0.58	0.065 ± 0.010
5	20.70 ± 0.76	0.650 ± 0.050	19.20 ± 0.43	0.534 ± 0.040	18.50 ± 0.90	0.487 ± 0.080	13.80 ± 0.86	0.151 ± 0.050
6	23.00 ± 0.79	0.848 ± 0.090	21.50 ± 0.48	0.687 ± 0.030	20.40 ± 0.93	0.634 ± 0.070	16.30 ± 1.01	0.230 ± 0.070
7	24.40 ± 0.78	0.025 ± 0.100	23.00 ± 0.56	0.818 ± 0.050	21.50 ± 0.96	0.739 ± 0.090	18.00 ± 1.07	0.355 ± 0.080
8	26.10 ± 0.78	0.318 ± 0.120	25.10 ± 0.60	0.979 ± 0.080	23.20 ± 1.11	0.958 ± 0.110	19.00 ± 1.07	0.449 ± 0.080
9	29.15 ± 0.52	1.880 ± 0.110	28.40 ± 0.50	1.487 ± 0.090	27.60 ± 0.97	1.397 ± 0.160	19.50 ± 1.17	0.538 ± 0.090

Abbreviations: Cc- *Chaetoceros calcitrans*, Ig- *Isochrysis galbana*,
Ns- *Nanochloropsis salina*, Sc- *Skeletonema costatum*,
Tg- *Tetraselmis gracilis*

Table 22. **Morphometric relationships for *P. fucata* spat following log transformation of values for Dorso Ventral Margin (DVM) and wet weight (WW, g) (n = 27).**

Microalgae	Regression equation	r^2
Cc + Ig	WW = 1.93 DVM – 5.86	0.95
Cc + Sc	WW = 2.25 DVM – 7.27	0.97
Ig + Sc	WW = 2.14 DVM – 6.89	0.94
Cc + Ig + Ns	WW = 2.21 DVM – 7.10	0.97
Cc + Ig + Sc	WW = 2.41 DVM – 7.82	0.96
Ns + Sc + Tg	WW = 2.18 DVM – 7.39	0.95
Cc + Ig + Ns + Sc + Tg	WW = 2.56 DVM – 8.19	0.97

Abbreviations: Cc- *Chaetoceros calcitrans,* Ig- *Isochrysis galbana,*
Ns- *Nanochloropsis salina,* Sc- *Skeletonema costatum,*
Tg- *Tetraselmis gracilis*

Table 23. The growth of spat of *Pinctada fucata* in a 56 day period when fed different rations by cell density of microalgae

Diet	Initial shell length (mm)	Final shell length (mm)	% Growth increase
Cc + Ig	4.00 ± 0.00	25.50 ± 0.83	537.50
Cc + Sc	5.75 ± 0.09	27.00 ± 0.71	369.56
Ig + Sc	5.00 ± 0.10	24.60 ± 0.82	392.00
Cc + Ig + Ns	5.30 ± 0.17	28.40 ± 0.05	435.84
Cc + Ig + Sc	5.15 ± 0.20	27.60 ± 0.97	435.92
Ns + Sc + Tg	5.25 ± 0.19	19.50 ± 1.17	271.42
Cc + Ig + Ns + Sc + Tg	5.35 ± 0.19	29.15 ± 0.52	444.85

Abbreviations: Cc- *Chaetoceros calcitrans*, Ig- *Isochrysis galbana*, Ns- *Nanochloropsis salina*, Sc- *Skeletonema costatum*, Tg- *Tetraselmis gracilis*

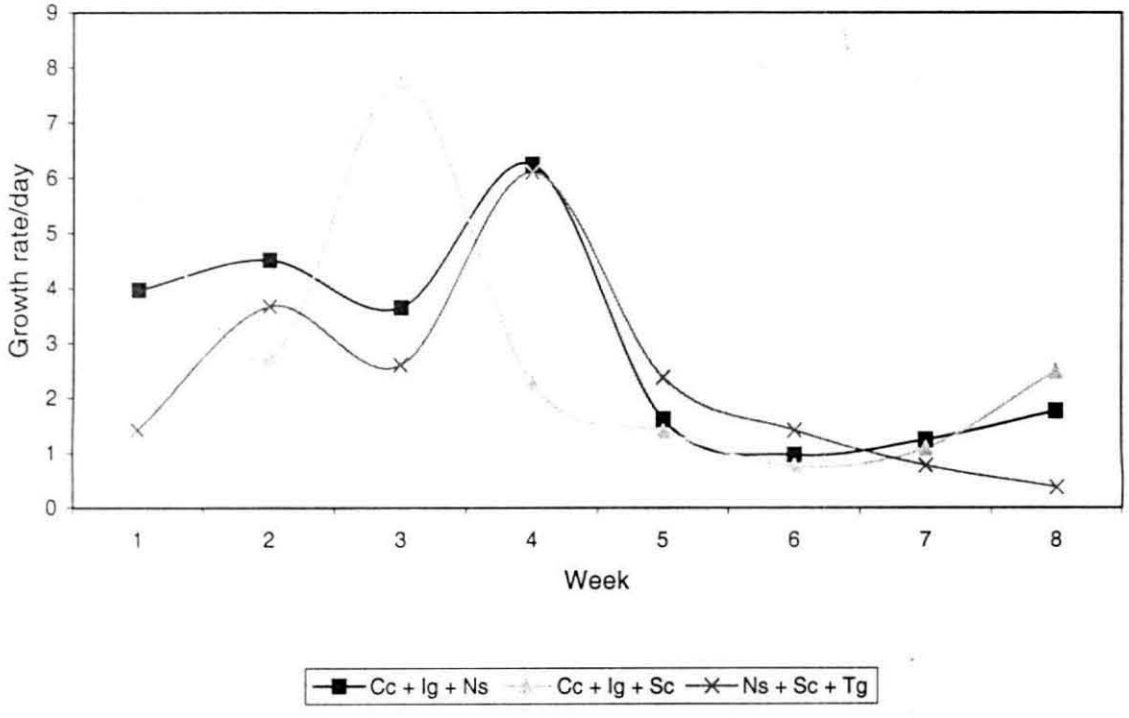

Figure 15. Growth rate of P. fucata spat in DVM (mm) when fed with different combinations of microalgae

Abbreviations:C.c.- *C. calcitrans,* I.g.- *I. galbana,* N.s.- *N. salina,* S.c.- *S.costatum,* T.g.- *T. gracilis*

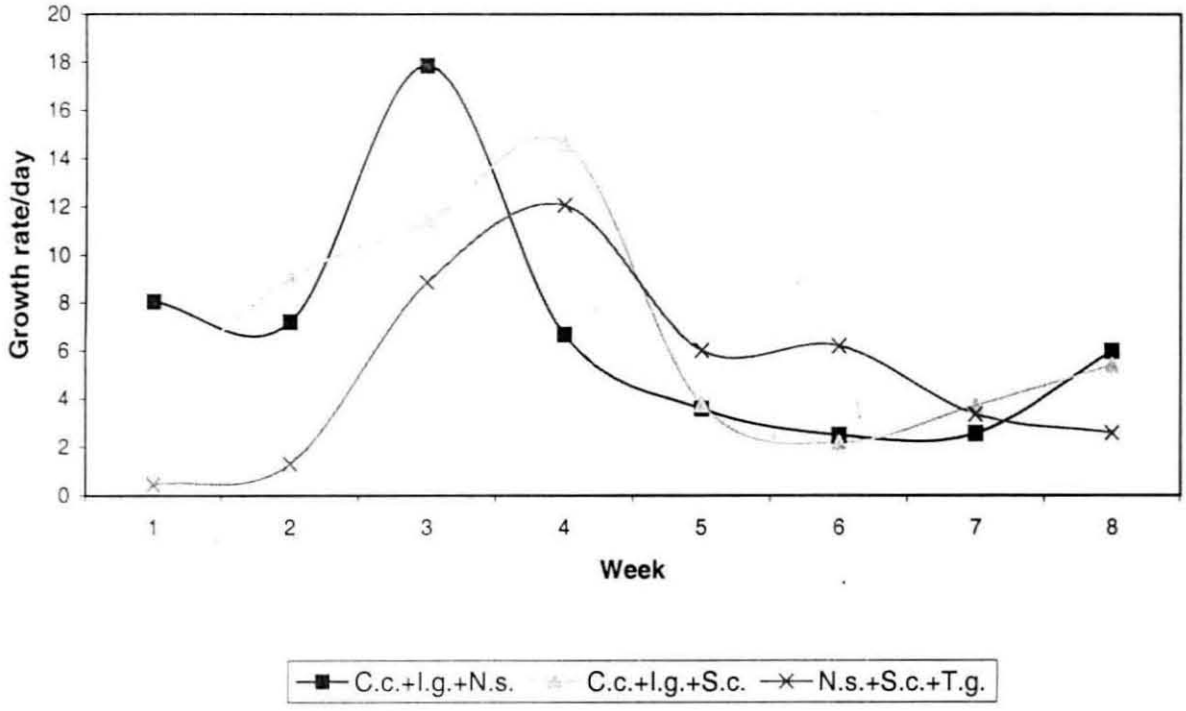

Figure 16. Growth rate of *P. fucata* spat in wet weight (WW, g) when fed with different combinations of microalgae

Abbreviations:C.c.-*C. calcitrans*, I.g.- *I. galbana*, N.s.-*N. salina*, T.g.- *T. gracilis*

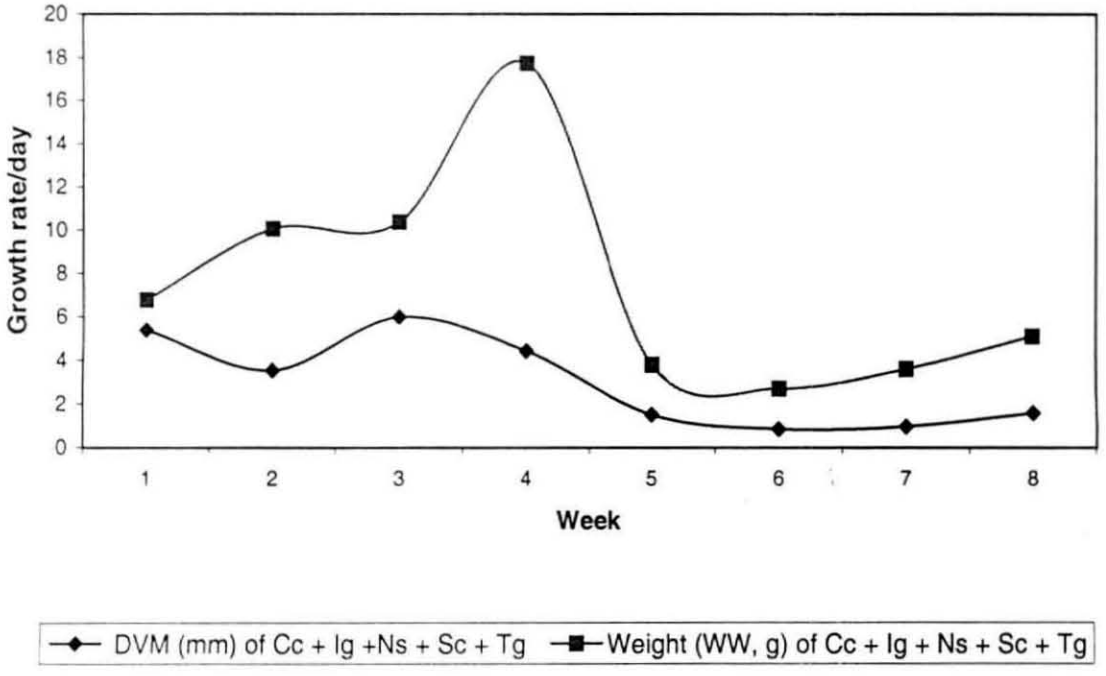

Figure 17. Growth rate of *P. fucata* spat in DVM (mm) and Wet Weight (WW, g) when fed with combination of microalgae

Abbreviations: Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costaum*, Tg- *T. gracilis*

Comparison of spat growth between combinations

A one-way ANOVA showed significant ($P<0.01$) differences between treatments for both DVM and WW (Table 24). The relatively low growth of the two species combinations of *C. calcitrans* + *I. galbana* and *I. galbana* + *S. costatum* and the three species diet of *N. salina* + *S. costatum* + *T. gracilis* resulted in significant differences with the other groups (Tables 20 & 21). Treatment comparisons between single species and combinations of microalgae also indicated significant ($P<0.01$) differences (Tables 28 & 29).

Table 24. A one-way ANOVA between combined species of microalgae for DVM (mm) and wet weight (WW, g) of *P. fucata*

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	944.28	6	157.38	3.08	<0.01
Error	9301.16	182	51.11		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	4.05	6	0.66	4.19	<0.01
Error	29.367	182	0.161		

Table 25. Combined species of microalgal comparisons between treatments for DVM based on ANOVA tables

Micro-algae	Cc+Sc	Ig+Sc	Cc+Ig+Ns	Cc+Ig+Sc	Ns+Sc+Tg	Cc+Ig+ Ns+Sc+Tg
Cc+Ig	n.s.	n.s.	sig	sig	n.s.	sig
Cc+Sc		n.s.				n.s.
Ig+Sc						sig
Cc+Ig+Ns	n.s.	sig		n.s.	sig	n.s.
Cc+Ig+Sc	n.s.	sig			sig	n.s.
Ns+Sc+ Tg	n.s.	n.s.				sig

Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

n.s.- not significant

sig - significant

Table 26. **Combined species of microalgal comparisons between treatments for WW based on ANOVA tables**

Micro-algae	Cc+Sc	Ig+Sc	Cc+Ig+Ns	Cc+Ig+Sc	Ns+Sc+Tg	Cc+Ig+ Ns+Sc+Tg
Cc+Ig	n.s.	n.s.	n.s.	n.s.	sig	sig
Cc+Sc		n.s.				sig
Ig+Sc						sig
Cc+Ig+Ns	n.s.	sig		n.s	sig	n.s.
Cc+Ig+Sc	n.s.	n.s.			sig	n.s.
Ns+Sc+ Tg	n.s.	n.s.				sig

Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

n.s.- not significant

sig - significant

Table 27. **A one-way ANOVA between single and combined species of microalgae for DVM (mm) and wet weight (WW, g) of *P. fucata***

A. DVM (mm)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	2098.38	11	190.76	4.54	<0.01
Error	13119.26	312	42.05		
B. Wet weight (WW, g)					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	8.29	11	0.75	6.89	<0.01
Error	34.14	312	0.11		

Table 28. Single and combined species of microalgal comparisons between treatments for DVM based on ANOVA tables

Microalgae	2	3	4	5	6	7	8	9	10	11	12
1	sig	sig	n.s	sig	sig	n.s.	n.s.	sig	sig	sig	n.s.
2					n.s.	sig	sig	n.s.	n.s.	n.s.	sig
3	n.s				n.s.	sig	sig	n.s.	n.s.	n.s.	sig
4	n.s	n.s			sig	sig	n.s.	n.s.	n.s.	sig	n.s.
5	n.s	n.s	n.s		n.s.	sig	sig	n.s.	n.s.	n.s.	sig
6								n.s.	n.s.	n.s.	sig
7					sig			sig	sig	sig	n.s.
8					sig	n.s.		sig	sig	sig	n.s.
9											sig
10								n.s.			sig
11								n.s.	n.s		sig

Abbreviations: 1- *C. calcitrans*, 2- *I. galbana*, 3- *N. salina*, 4- *S. costatum*,
5- *T. gracilis*, 6- *C. calcitrans* +*I. galbana*,
7- *C. calcitrans* + *S. costatum*, 8- *I. galbana* + *S. costatum*,
9- *C. calcitrans* +*I. galbana* + *N. salina*,
10- *C. calcitrans* +*I. galbana* + *S. costatum*,
11- *N. salina* + *S. costatum* + *T. gracilis*,
12- *C. calcitrans* +*I. galbana* + *N. salina*
n.s.- not significant
sig - significant

Table 29. Single and combined species of microalgal comparisons between treatments for WW based on ANOVA tables

Microalgae	2	3	4	5	6	7	8	9	10	11	12
1	sig	n.s	n.s	sig	sig	n.s.	n.s.	sig	sig	sig	sig
2					n.s.	sig	sig	n.s.	n.s.	n.s.	n.s.
3	sig				sig	sig	n.s.	sig	sig	sig	n.s.
4	n.s	n.s			sig	sig	sig	n.s.	n.s.	sig	n.s.
5	n.s	n.s	n.s		n.s.	sig	sig	n.s.	n.s.	n.s.	n.s.
6								n.s.	n.s.	n.s.	n.s.
7					sig			sig	sig	sig	sig
8					sig	n.s.		sig	sig	sig	sig
9											n.s.
10								n.s.			n.s.
11								n.s.	n.s.		n.s.

Abbreviations: 1- *C. calcitrans*, 2- *I. galbana*, 3- *N. salina*, 4- *S. costatum*,
5- *T. gracilis*, 6- *C. calcitrans* +*I. galbana*,
7- *C. calcitrans* + *S. costatum*, 8- *I. galbana* + *S. costatum*,
9- *C. calcitrans* +*I. galbana* + *N. salina*,
10- *C. calcitrans* +*I. galbana* + *S. costatum*,
11- *N. salina* + *S. costatum* + *T. gracilis*,
12- *C. calcitrans* +*I. galbana* + *N. salina*
n.s.- not significant
sig - significant

4. 3. BIOCHEMICAL COMPOSITION OF MICROALGAE AND SPAT

The data on biochemical composition of the five species of algae are presented in Table 30. Protein and carbohydrate did not show wide variations while lipid content ranged from 6% in *T. gracilis* to 19% in *C. calcitrans*. Statistical analysis also revealed significant difference in the case of lipid content (Table 31). Significant differences are also noticed between individual species algae except in the case of *C. calcitrans* and *I. galbana* and between *S. costatum* and *T. gracilis* (Table 32). All biochemical parameters studied in pearl oyster spat show a decreasing trend with increase in stocking density (Table 33), with significant differences, $P<0.01$ (Table 34). Comparison between densities is also significant except in the case of phospholipid at low and moderate stocking density (Table 35).

Biochemical composition of spat fed with different algae singly and in combination are given in Table 36. The percentage of protein is high in spat fed with *C. calcitrans* and low in *T. gracilis*. Two species combination of algae also show high percentage of protein in spat except the combination of *I. galbana* and *S. costatum*. The three species combination also indicates high protein except the combination of *N. salina*, *S. costatum* and *T. gracilis*. The total lipid content of spat also indicates a decreasing trend from 8.3% in *C. calcitrans* to 3.9% dry weight in *T. gracilis*. Algal combination also shows lipid content is 8% except with the combination of *N. salina*, *S. costatum* and *T. gracilis*. The carbohydrate values also show a decrease from *C. calcitrans* to *T. gracilis*. All combinations show values around 20% carbohydrate. Maximum value has been recorded in the combination of the five species of algae together. Significant differences ($P<0.01$) have been noticed in all parameters when fed with single species of algae (Table 37). Comparison between single species indicated significant differences (Table 38).

ANOVA have also indicated significant differences ($P<0.01$) except for phospholipid when fed with combination of algae (Table 39).

Table 30. **Biochemical composition of microalgae spat in percentage dry weight**

Microalgae	Moisture (%)	Protein (% of dry weight)	Lipid (% of dry weight)	Carbohydrate (% of dry weight)
C. calcitrans	55 ± 2.0	28 ± 2.0	19 ± 1.0	5 ± 0.1
I. galbana	57 ± 3.0	23 ± 1.0	17 ± 2.0	4 ± 0.3
N. salina	56 ± 1.0	25 ± 3.0	12 ± 1.0	8 ± 0.2
S. costatum	55 ± 3.0	29 ± 1.0	8 ± 0.2	6 ± 0.1
T. gracilis	55 ± 1.0	26 ± 3.0	6 ± 0.1	7 ± 0.3

Table 31. A one-way ANOVA between microalgal biochemical composition in percentage dry weight

A. Protein					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	29.60	4	7.40	3.70	>0.01
Error	10.00	5	2.00		
B. Lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	250.40	4	62.60	31.30	<0.01
Error	10.00	5	2.00		
C. Carbohydrate					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	20.00	4	5.00	2.50	>0.01
Error	10.00	5	2.00		

Table 32. **Microalgal lipid composition comparisons based on ANOVA tables**

Microalga	*I. galbana*	*N. salina*	*S. costatum*	*T. gracilis*
C. calcitrans	n.s.	sig	sig	sig
I. galbana		sig	sig	sig
N. salina			sig	sig
S. costatum				n.s.

n.s.- not significant

sig - significant

Table 33. Biochemical composition of pearl oyster *P. fucata* spat

Density	Moisture (mg)	Ash (mg)	Protein (%dry wt)	Lipid (%dry wt)	Carbo hydrate (%dry wt)	Phospho lipid (%dry wt)	Neutral Lipid (%dry wt)
20/60 l	80.4 ± 1.0	16.5 ± 0.2	87.8 ± 3.0	8.3 ± 0.1	20.8 ± 0.2	2.1 ± 0.1	4.0 ± 0.1
40/60 l	79.9 ± 1.0	14.8 ± 3.0	82.2 ± 0.15	7.7 ± 0.3	15.7 ± 0.3	1.9 ± 0.4	3.6 ± 0.3
80/60 l	79.5 ± 2.0	12.9 ± 1.0	78.6 ± 0.3	6.9 ± 0.2	10.9 ± 0.2	1.7 ± 0.3	3.2 ± 0.1
160/60 l	78.2 ± 1.0	8.7 ± 0.2	70.5 ± 0.2	3.2 ± 0.1	2.8 ± 0.2	0.8 ± 0.3	1.4 ± 0.2

Table 34. A one-way ANOVA between biochemical composition of *P. fucata* spat reared at different densities

A. Protein					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	232.57	3	77.53	3780.25	<0.01
Error	0.08	4	0.02		
B. Lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	31.46	3	10.49	524.34	<0.01
Error	0.08	4	0.02		
C. Carbohydrate					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	351.54	3	117.18	5853.28	<0.01
Error	0.08	4	0.02		
D. Phospholipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	1.96	3	0.66	32.92	<0.01
Error	0.08	4	0.02		
E. Neutral lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	7.90	3	2.63	131.65	<0.01
Error	0.08	4	0.02		

Table 35. *P. fucata* spat biochemical composition comparisons reared at different densities based on ANOVA tables

A. Protein			
Density	**40/60 l**	**80/60 l**	**160/60 l**
20/60 l	Sig	sig	sig
40/60 l		sig	sig
80/60 l			sig
B. Lipid			
Density	**40/60 l**	**80/60 l**	**160/60 l**
20/60 l	Sig	sig	sig
40/60 l		sig	sig
80/60 l			sig
C. Carbohydrate			
Density	**40/60 l**	**80/60 l**	**160/60 l**
20/60 l	Sig	sig	sig
40/60 l		sig	sig
80/60 l			sig
D. Phospholipid			
Density	**40/60 l**	**80/60 l**	**160/60 l**
20/60 l	n.s.	sig	sig
40/60 l		n.s.	sig
80/60 l			sig
E. Neutral lipid			
Density	**40/60 l**	**80/60 l**	**160/60 l**
20/60 l	Sig	sig	sig
40/60 l		sig	sig
80/60 l			sig

n.s.- not significant

sig - significant

Table 36. Biochemical composition of pearl oyster, *P. fucata* spat fed with single algae and combinations of algae

Diet	Moisture (mg)	Ash (mg)	Protein (%dry wt)	Lipid (%dry wt)	Carbo hydrate (%dry wt)	Phospho lipid (%dry wt)	Neutral lipid (%dry wt)
Cc	80.4 ± 3.0	16.5 ± 1.0	87.8 ± 2.0	8.3 ± 0.3	20.8 ± 3.0	2.1 ± 0.3	4.0 ± 0.4
Ig	80.2 ± 1.0	14.8 ± 0.2	83.6 ± 3.0	7.9 ± 0.2	17.4 ± 2.0	1.7 ± 0.2	3.7 ± 0.3
Ns	80.2 ± 4.0	12.1 ± 3.0	82.9 ± 2.0	5.8 ± 0.2	15.2 ± 2.0	1.4 ± 0.1	2.7 ± 0.2
Sc	80.2 ± 3.0	11.4 ± 1.0	81.0 ± 4.0	4.6 ± 0.1	14.6 ± 1.0	1.0 ± 0.1	2.1 ± 0.2
Tg	80.0 ± 1.0	9.2 ± 0.2	78.8 ± 3.0	3.9 ± 0.1	12.7 ± 1.0	0.8 ± 0.1	1.7 ± 0.1
Cc+Ig	80.6 ± 2.0	16.5 ± 1.0	88.7 ± 3.0	8.4 ± 0.2	21.0 ± 3.0	1.9 ± 0.2	4.0 ± 0.4
Cc+Sc	80.5 ± 1.0	16.5 ± 3.0	88.5 ± 2.0	8.4 ± 0.2	20.9 ± 3.0	1.9 ± 0.2	4.0 ± 0.4
Ig+Sc	80.3 ± 4.0	15.7 ± 2.0	85.2 ± 1.0	8.0 ± 0.1	18.5 ± 2.0	1.8 ± 0.2	3.8 ± 0.3
Cc+Ig+Ns	80.8 ± 1.0	16.6 ± 3.0	89.4 ± 2.0	8.5 ± 0.3	21.7 ± 3.0	1.9 ± 0.2	4.0 ± 0.4
Cc+Ig+Sc	80.7 ± 1.0	16.5 ± 2.0	89.1 ± 3.0	8.5 ± 0.3	21.2 ± 3.0	1.9 ± 0.2	4.0 ± 0.4
Ns+Sc+Tg	80.3 ± 2.0	13.9 ± 3.0	83.4 ± 2.0	6.7 ± 0.1	16.8 ± 2.0	1.6 ± 0.2	3.1 ± 0.3
Cc+Ig+Ns+Sc+Tg	81.0 ± 3.0	16.7 ± 1.0	89.9 ± 2.0	8.6 ± 0.3	22.5 ± 3.0	2 ± 0.3	4.1 ± 0.4

Abbreviations: Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

Table 37. A one-way ANOVA between biochemical composition of *P. fucata* spat when fed with single species of microalgae

A. Protein					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	89.78	4	22.45	1197.08	<0.01
Error	0.09	5	0.02		
B. Lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	30.52	4	7.63	381.59	<0.01
Error	0.10	5	0.02		
C. Carbohydrate					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	76.78	4	19.19	958.86	<0.01
Error	0.10	5	0.02		
D. Phospholipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	2.20	4	0.55	27.50	<0.01
Error	0.10	5	0.02		
E. Neutral lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	7.90	4	1.98	98.79	<0.01
Error	0.10	5	0.02		

Table 38. *P. fucata* spat biochemical composition comparisons when fed with single species of microalgae based on ANOVA tables

A. Protein				
Microalga	*I. galbana*	*N. salina*	*S. costatum*	*T. gracilis*
C. calcitrans	sig	sig	sig	sig
I. galbana		sig	sig	sig
N. salina			sig	sig
S. costatum				sig
B. Lipid				
Microalga	*I. galbana*	*N. salina*	*S. costatum*	*T. gracilis*
C. calcitrans	sig	sig	sig	sig
I. galbana		sig	sig	sig
N. salina			sig	sig
S. costatum				sig
C. Carbohydrate				
Microalga	*I. galbana*	*N. salina*	*S. costatum*	*T. gracilis*
C. calcitrans	sig	sig	sig	sig
I. galbana		sig	sig	sig
N. salina			sig	sig
S. costatum				sig

n.s.- not significant

sig - significant

Table 38. *P. fucata* **spat biochemical composition comparisons when fed with single species of microalgae based on ANOVA tables**

D. Phospholipid				
Microalga	***I. galbana***	***N. salina***	***S. costatum***	***T. gracilis***
C. calcitrans	sig	sig	sig	sig
I. galbana		n.s.	sig	sig
N. salina			sig	sig
S. costatum				n.s.
E. Neutral lipid				
Microalga	***I. galbana***	***N. salina***	***S. costatum***	***T. gracilis***
C. calcitrans	n.s.	sig	sig	sig
I. galbana		sig	sig	sig
N. salina			sig	sig
S. costatum				sig

n.s.- not significant

sig - significant

Algal combinations have also shown significant variations in protein content except in the case of the combination of *C. calcitrans* and *I. galbana/S. costatum* and the three species combination of *C. calcitrans*, *I. galbana* and *N. salina* and *S. costatum* (Table 40). In the case of lipids, the relatively low values for *N. salina*, *S. costatum* and *T. gracilis* have resulted in significant differences when compared with feeds containing *C. calcitrans* and *I. galbana*. Similarly, low carbohydrate values in *S. costatum* and *T. gracilis* also resulted in significant variations when they were used as feeds. The use of the microalga *T. gracilis* has resulted in low phospholipid in spat contributing to significant differences when compared with other combinations.

A comparative picture of the microalgae and their biochemical constituents are presented in Figure 18. There is no major variation in the protein content with a range from 22.5 to 27.5% dry weight. The lipid content, however, shows a clear decreasing trend from *C. calcitrans* to the lowest value in *T. gracilis* value. The percentage of carbohydrate was lower than that of both protein and lipid and ranged from only 4 to 7%. *N. salina* indicated the maximum percentage of carbohydrate. The protein content of spat at all stocking densities were high with values over 70% (Fig. 19) and it showed a decreasing trend with increase in density. This decrease in values with increase in density was also noticed in the case of carbohydrate. Phospholipid content was very low, less than 1%. The biochemical composition of spat when fed with different combinations of algae also showed similar trends (Figs. 20 & 21).

Table 39. A one-way ANOVA between biochemical composition of *P. fucata* spat when fed with combination of microalgae

A. Protein					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	72.10	6	12.02	566.75	<0.01
Error	0.15	7	0.02		
B. Lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	5.39	6	0.90	44.93	<0.01
Error	0.14	7	0.02		
C. Carbohydrate					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	47.83	6	7.97	400.97	<0.01
Error	0.14	7	0.02		
D. Phospholipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	0.19	6	0.03	1.62	>0.01
Error	0.14	7	0.02		
E. Neutral lipid					
Source of variation	**SS**	**df**	**MS**	**F**	**P**
Treatment	1.43	6	0.24	11.95	<0.01
Error	0.14	7	0.02		

Table 40. *P. fucata* spat biochemical composition comparisons when fed with combination of microalgae based on ANOVA tables

A. Protein						
Microalga	Cc+Sc	Ig+Sc	Cc+Ig+Ns	Cc+Ig+Sc	Ns+Sc+Tg	Cc+Ig+Ns+Sc+Tg
Cc+Ig	n.s.	sig	sig	sig	sig	sig
Cc+Sc		sig	sig	sig	sig	sig
Ig+Sc			sig	sig	sig	sig
Cc+Ig+Ns				n.s.	sig	sig
Cc+Ig+Sc					sig	sig
Ns+Sc+Tg						sig
B. Lipid						
Microalga	Cc+Sc	Ig+Sc	Cc+Ig+Ns	Cc+Ig+Sc	Ns+Sc+Tg	Cc+Ig+Ns+Sc+Tg
Cc+Ig	n.s.	sig	n.s.	n.s.	sig	n.s.
Cc+Sc		sig	n.s.	n.s.	sig	n.s.
Ig+Sc			sig	sig	sig	sig
Cc+Ig+Ns				n.s.	sig	n.s.
Cc+Ig+Sc					sig	n.s.
Ns+Sc+Tg						sig

Abbreviations: Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

n.s.- not significant

sig - significant

Table 40. *P. fucata* spat biochemical composition comparisons when fed with combination of microalgae based on ANOVA tables

C. Carbohydrate						
Microalga	**Cc+Sc**	**Ig+Sc**	**Cc+Ig+ Ns**	**Cc+Ig+ Sc**	**Ns+Sc+ Tg**	**Cc+Ig+Ns+ Sc+Tg**
Cc+Ig	n.s.	sig	sig	n.s.	sig	sig
Cc+Sc		sig	sig	n.s.	sig	sig
Ig+Sc			sig	sig	sig	sig
Cc+Ig+Ns				sig	sig	sig
Cc+Ig+Sc					sig	sig
Ns+Sc+Tg						sig
D. Neutral lipid						
Microalga	**Cc+Sc**	**Ig+Sc**	**Cc+Ig+ Ns**	**Cc+Ig+ Sc**	**Ns+Sc+ Tg**	**Cc+Ig+Ns+ Sc+Tg**
Cc+Ig	n.s.	n.s.	n.s.	n.s.	sig	n.s.
Cc+Sc		n.s.	n.s.	n.s.	sig	n.s.
Ig+Sc			n.s.	n.s.	sig	n.s.
Cc+Ig+Ns				n.s.	sig	n.s.
Cc+Ig+Sc					sig	n.s.
Ns+Sc+Tg						sig

Abbreviations: Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

n.s.- not significant

sig - significant

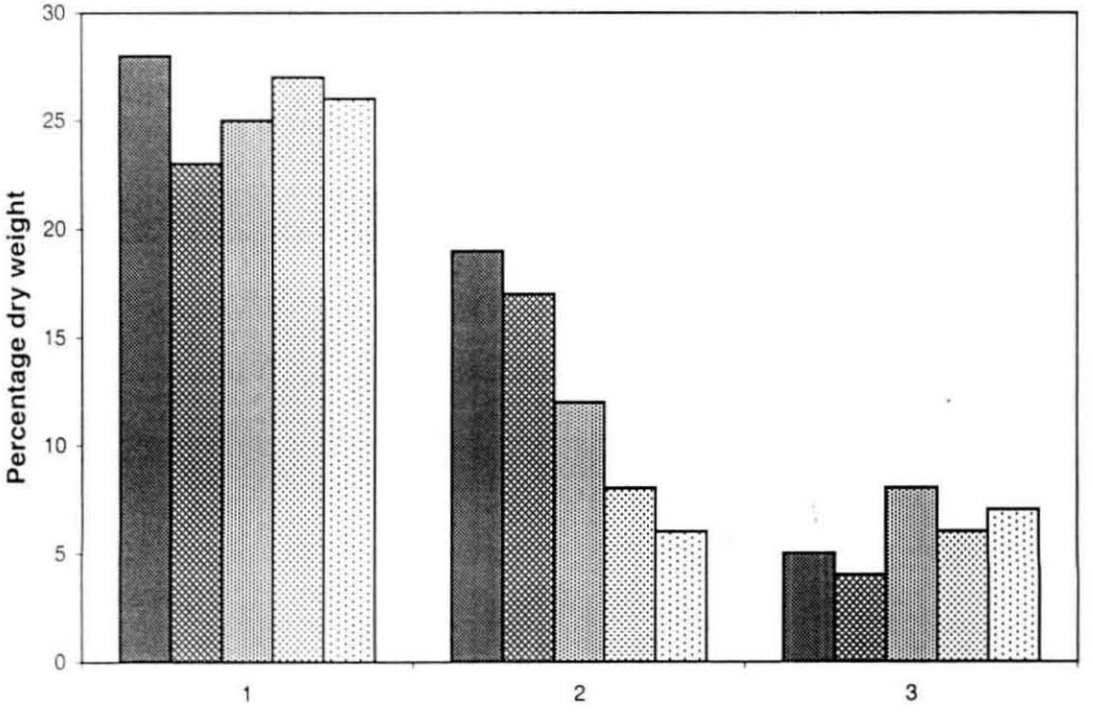

Figure 18. Biochemical composition of microalgae

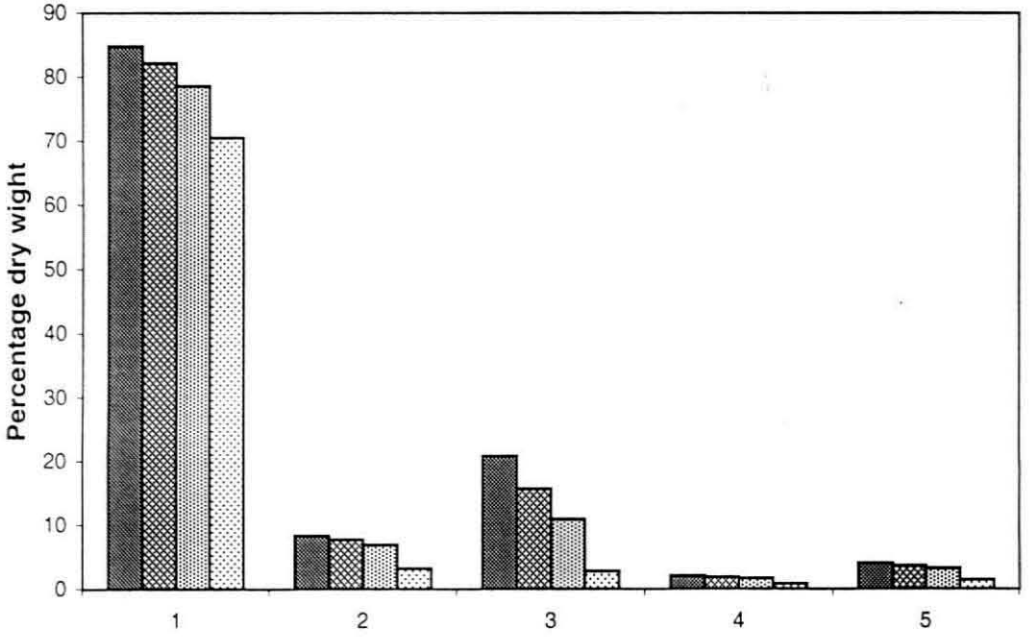

Figure 19. Biochemical composition of spat

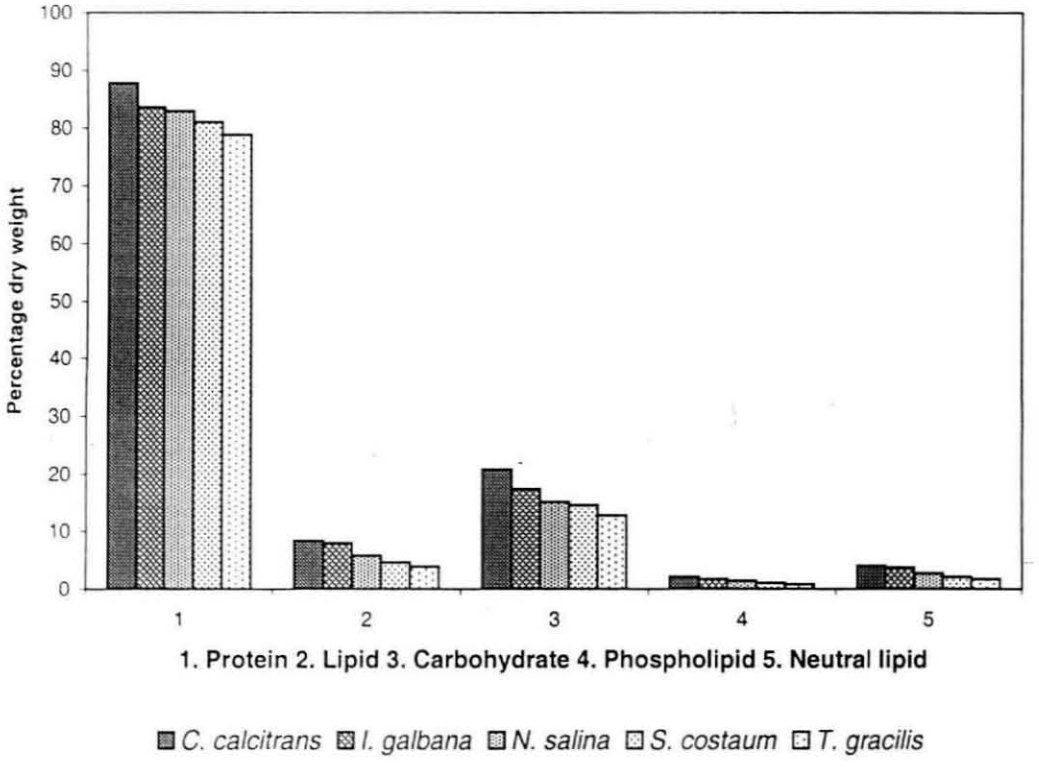

Figure 20. Biochemical composition of spat when fed with single species of microalgae

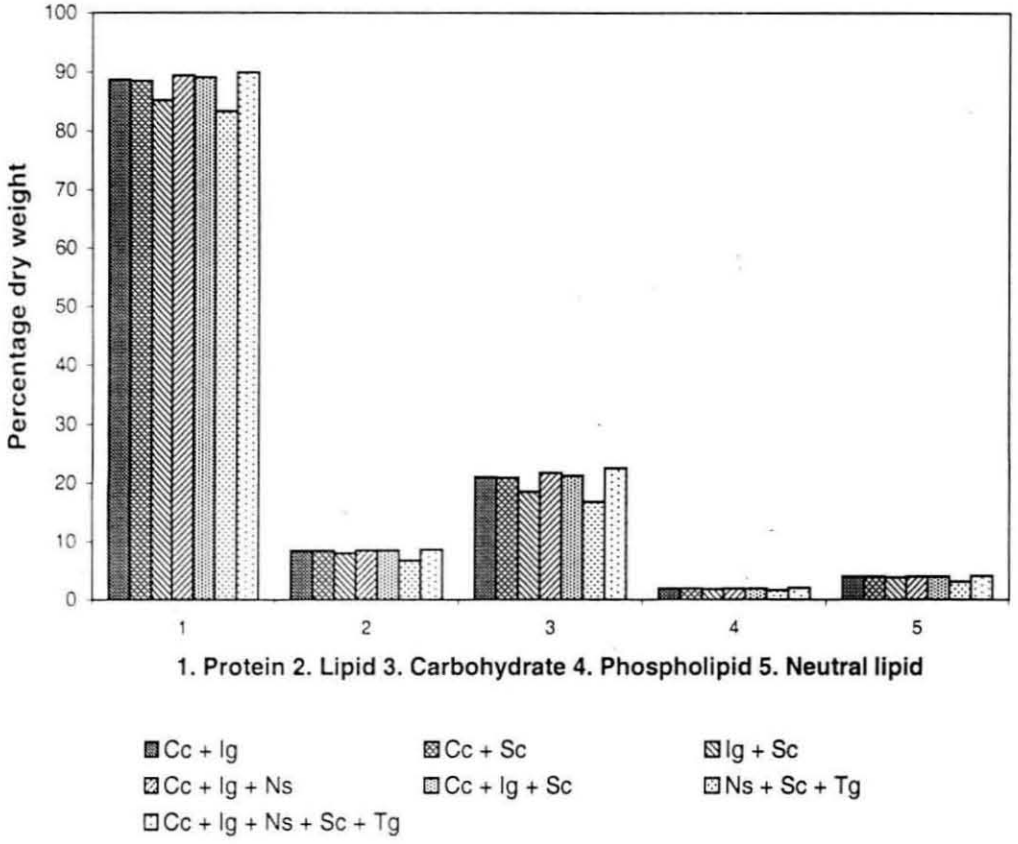

Figure 21. Biochemical composition of spat when fed with combinations of microalgae

Abbreviations: Cc- *C. calcitrans*, Ig- *I. galbana*, Ns- *N. salina*, Sc- *S. costatum*, Tg- *T. gracilis*

5. DISCUSSION

5. 1. SPAT DENSITY

An important problem faced in the rearing of bivalve spat is the determination of the optimum density of stocking at which the survival and growth rate will be highest. Rose and Baker (1994) have observed significant difference in growth of spat of *Pinctada maxima* at densities of 4 and 25 spat/100cm^2. In the present study the spat of *P. fucata* showed optimum growth at density of 67 spat/m^2 . Chellam *et al.* (1987) have reported optimum growth of oysters stocked in net cages of 40 x 40 cm at a density of 125 oysters in the size group of 35-45 mm, 100 oysters in the size group 45-55 mm and 75 oysters in the size group 55-60 mm. Alagarswami *et al.* (1983) have found that the spat of *P. fucata* exhibit variation in growth within a batch. Differential growth rate of spat have been attributed to presence of slower-developing spat in the group, inappropriate preparation of collectors and improper choice of algal diet (Rose and Baker, 1994).

Southgate and Beer (1997) have reported similar results for *P. margaritifera* as in the present study. They observed that pearl oysters held at a density of 20 per pearl net had greater dorsoventral height (DVH), and wet weight (WW) than those held at any other densities. As observed in the present study, there was a progressive decline in mean DVH and WW with increasing stocking density and spat held at densities of 20 and 50 per net had significantly greater growth than those held in higher densities. Large variation in the growth rate of juvenile *P. maxima* and *P. margaritifera* cohorts has been reported for both wild (Scoones, 1990) and hatchery cultured juveniles (Alagarswami *et al.*, 1989; Rose and Baker, 1994). A similar variation in spat size has recorded in the present study. At the 56 days of age, the largest individual had a DVM of 32 mm, while the smallest had DVM measurement of 17 mm.

Achieving maximum growth rates in the nursery phase of pearl oyster culture reduces the time required to rear to implantation size for pearl production. Scoones (1990) reported that slower growing *P. maxima* juveniles in Western Australia required 30 months to reach commercial size compared with 18 months for the rapid growers. Smaller or slower growing pearl oyster juveniles require more frequent maintenance and have longer non productive culture period. In onshore culture system for *P. fucata* at Visakhapatnam, India, minimum commercial size is reached in six months (Rao & Devaraj, 1996). In the present study *P. fucata* attained 26.75 mm from 8.0 mm within 56 days.

The spat of *P. fucata* in the present study have shown growth rates higher to those reported for *P. margaritifera* and *P. maxima* in other studies. Alagarswami *et al.* (1989) have reported a daily DVH growth rate of 0.4 mm/day for hatchery reared *P. margaritifera* spat. Coeroli *et al.* (1984) have reported that spat held in suspended culture at 3 m reached a DVH of 8-10 mm after 3 months and 40-50 mm after 6 months. In Solomon Islands, Friedman and Bell (1996) have reported a mean DVH of 32.4 mm in a period of six months and Southgate and Beer (1997) have found similar growth rate of 40 mm after 7.5 months.

A significant aspect of the results in the present study is the 100% survival rate observed even in higher densities. The survival rate of *P. margaritifera* in nursery trial in sea ranges from 15-17% (Alagarswami *et al.*, 1989) to 29-34% (Southgate and Beer, 1997). However, Rose and Baker (1994) have reported a survival rate of 88-99% in the case of *P. maxima* in spat reared in downwellers and in sea cages. But they caution that rearing spat in downwellers for 5 months is not cost-effective even though survival is high.

In each of the three experiments, individual spat growth decreased with increasing density, probably because of competition for food. Hadley and Manzi (1984) have concluded that food was the growth-limiting factor for clams (*Mercenaria mercenaria*) stocked at a range of densities in a raceway

nursery system. A variety of criteria could be used to assess optimum stocking density (Holliday *et al.*, 1991). The higher growth rate at low densities enhances the value of individual oysters. However, the production per unit area may be relatively low. Optimum density may also be influenced by survival rate, although in all the present experiments, survival rates were 100% and unaffected by density. The choice of stocking density may also be based on economic considerations (Maguire and Leedow, 1983). Factors like strong currents, large tidal cycles and fluctuations in phytoplankters in sea influence survival of spat in traditional nursery system in salinity (Tanaka and Kumeta, 1981). However, these factors do not adversely affect spat growth in onshore culture of pearl oysters.

A statistically precise correlation could be calculated between length and individual weight of spat with length as a good index of growth. Paynter and Dimichele (1990) have reported high coefficient of determination between length and weight of *Crassostrea virginica* grown in trays in Chesapeake Bay. The length weight relationship indicates a linear increase throughout the growth period and a correlation with an expected logarithmic increase in tissue weight. Although isometric growth was observed at the different stocking densities with **b** values significantly higher or lower than 3.0, it shows that length is a good indicator of growth in the spat of *P. fucata*.

Narasimham (1988 a, b) observed sigmoid curve for the growth of *Anadora granosa* and *A.rhombea* at Kakinada Bay. Theisen (1973) observed that the growth curve of *Mytilus edulis* is of sigmoid form and that the von Bertalanffy growth equation gave the best fit to the observed length data pertaining to above $1/3^{rd}$ of the maximum length. He concluded that the sigmoid growth curve is common to most lamellibranches and hence the von Bertalanffy growth equation should not be used to indicate the growth of lamellibranches. The growth curves for length and weight of many organisms are sigmoid and among the bivalves sigmoid growth curves were observed by Stevenson and Dickie (1954), Ansell and Parlukar (1978) and Broom (1982). These studies however pertain to natural conditions where growth is influenced by the salinity and temperature of the water and the availability of

the food. In experimental conditions fluctuations in environmental parameters are minimum and food is supplied continuously, a deviation from the sigmoid growth pattern appear to be possible.

5. 2. ALGAL CONCENTRATION AND NUTRITIVE VALUE OF MICROALGAE

Except for *I. galbana* and *S. costatum*, the concentration of 60 cells/ µl resulted in maximum growth of spat in single species diets. Growth performance is directly correlated with food availability or the ration offered. The amount of energy acquired for growth by pearl oyster spat depends directly on the quantity of food available in suspension. Winter (1978), however, is of the opinion that the amount of algae filtered out within a wide range of concentrations is more or less constant. The high ingestion rate with increase in concentration of algae is a typical feature of juvenile bivalves and is caused by a partial lack of regulation of the ingestion rate which will influence growth rate (Beiras *et al.*, 1993). When the concentration of food is increased, the ingested food is not absorbed and is ejected as faeces. The low absorption efficiencies as indicated by reduced growth might also be due to unsuitable or indigestible food, to an abnormal physiological state of the spat or to experimental errors (Beiras *et al.*, 1993). They further explain that the direct correlation between absorbed food and growth is due to the fact that metabolic expenditure at high and medium food rations remain steady within routine levels. On the other hand, low food concentrations of about 15 and 30 cells/µl, supporting low growth, causes a reduced metabolic expenditure.

The growth exponent **b** also shows a decreasing trend with increase in algal cell concentration. Widdows (1978) also has found reduced regression slopes for *M. edulis* at high ration level as a result of an increase in the rate of oxygen consumption by smaller individuals. Schulte (1975) has observed high filtration rates in mussels at low concentrations, while at very high concentrations of algae the filtration activity dropped to very low values. The decreasing growth rates observed in the present study with increase in algal concentration suggests that the pearl oyster is not adapted for efficient

feeding at high food concentrations above 60 cells/μl. This may be probably due to reduction in filtering efficiency and/or pumping rate and increase in production of pseudofaeces. Tenore and Dunstan (1973) have observed similar results in the clam, *Mercenaria mercenaria*, but feeding rates of the mussel, *Mytilus edulis* and oyster, *Crassostrea virginica* were constant at higher food concentration levels.

Winter and Langton (1975) have shown for *Mytilus edulis* that growth, measured as an increase in dry meat weight, is a direct function of the quantity of food ingested up to an optimum level. As this optimum is exceeded, there is a rapid decrease in the quantity of food assimilated and an increase in the production of pseudofaeces. The quantity of phytoplankton consumed increases in proportion to the algal cell concentration, reaching a plateau where it is constant and independent of the particle concentration, and finally, decreasing with any further increase in algal cell density. From the present data on the amount of phytoplankton consumed, it is clear that the plateau is at 60 cells/μl in the case of *C. calcitrans*, *N. salina* and *T. gracilis*. In the case of *I. galbana* and *S. costatum*, it is earlier at 15 cell/μl and 30 cells/μl respectively. Langton *et al.* (1977) observed that the plateau is not reached in the case of the clam, *Tapes japonica* fed at three algal cell densities. Langton and Mckay (1976) have found that at two food levels, 120 and 180 cells/μl, discontinuous feeding gave consistently higher growth. In the present study, spat were fed twice daily with a gap of 8 hrs between feeding. The spat were large enough to filter all the water in the tank at least once before the commencement of the next feeding period. Filtration efficiency may also be greater during discontinuous feeding, possibly because the higher cell concentration introduced to clean tanks may stimulate filtration rate, and this, in turn, could result in net gain to the spat in terms of consumption. In closed recirculating system where feeding conditions are not constant, there may be difference in feeding efficiency so that spat fed discontinuously consume more food per day than those fed continuously (Langton and McKay, 1976).

The optimum algal ration for growth of *P. fucata* in the present study agrees with studies conducted in *M. mercenaria* (Coutteau, *et al.*, 1994) and *O. edulis* (Walne and Spencer, 1974). The daily ration reported in this study represent offered rations, which do not correspond to ingested rations, and part of the unconsumed algae may settle or was discarded during water renewal. Also part of the filtered algae may have been rejected as pseudofaeces due to the exposure of the oysters to higher concentrations (e. g. at 90 cells/μl).

The two species diet of *C. calcitrans* and *I. galbana* showed a better growth rate than when fed as individual diets. Helm and Laing (1987) have reported a similar observation on *Crassostrea gigas* and *C. rhizophorae* larvae. They suggest that *Isochrysis* may lack in a component or components vital to normal growth and development. Generally, *I. galbana* has been regarded as a satisfactory food for bivalve larvae (Loosanoff and Davis, 1963). Although, it is one of the better food species tested for *P. fucata* at lower concentrations, a mixture along with *C. calcitrans* has shown higher growth rates. Long duration nutrition trials by Wilson (1978) has provided information that points to a nutritional deficiency developing in *C. gigas* larvae fed solely on *I. galbana*. *C. calcitrans*, on the other hand, is a very good food for *Crassostrea* spp. Larvae as well as for clams (Helm and Laing, 1987). They attributed this good growth to a higher percentage of 20: 5 ω3 fatty acid in the total lipid content of *C. calcitrans*. The combination of *C. calcitrans* and *I. galbana* as a feed for *P. fucata* spat seems to strike the correct balance of components for optimal growth. Therefore, it is not only the presence or absence of certain essential components in the diet, but in attaining the correct balance between them, which will promote growth. The same also holds good for other combinations of three species diets or a combination of all the five algal species which showed remarkable growth.

Taylor *et al.* (1997) have stressed the importance of experimental testing of algal diets for bivalve spat rather than sole reliance on published nutritional values. Contrary to expectations, they observed low growth rates of *P. maxima* spat fed on *C. calcitrans* and *Pavlova lutheri*. They attributed this

to the nutritional values of algae, which vary according to its growing conditions.

Growth rates of algae produced under routine culture conditions differ from time to time, and this alters their food value. The amount of natural food present in filtered sea water is also an important, but often overlooked factor influencing the growth of oyster larvae (Nell and O' Connor, 1991). Another important source of carbon and nitrogen is the naturally available bacterioplankton of size less than 2.5μ that meets the dietary requirements of filter-feeding organisms (Lucas *et al.*, 1987). *P. fucata* spat showed low growth rates when fed on *T. gracilis* either singly or in combination. Epifanio (1979) has ascribed poor performance of a related species, *T. suecia* to the difficulties met by spat in digesting the theca of this alga. Undoubtedly, a mixed diet will produce better growth in *P. fucata*, as it has been previously demonstrated with other bivalves (Whyte *et al.*, 1989; Fidalgo *et al.*, 1994; Taylor *et al.*, 1997). Shumway *et al.* (1985) have recognized three mechanisms of selecting suspended particles in filter feeding bivalves. They are: (a) preferential clearance on the ctenidia; (b) preingestive selection on the labial palps (rejection in the pseudofaeces) and (c) differential absorption in the gut. The differential growth rates observed in *P. fucata* fed on mixed algal diets, points to a possibility of selective removal of particular components, and ingestion of materials that are quantitatively more important. *P. fucata* may also have the ability to ingest preferentially various types of organic material and to reject other particles as pseudofaeces as demonstrated in the case of American oyster, *Crassostrea virginica* (Newell and Jordan, 1983).

Empirical studies of the relationship between ration, size and growth of bivalves in small sized spat are scarce, although the juvenile stages are the largest consumers of cultured algae in commercial hatchery operations. Coutteau *et al.* (1994) suggest that most equations describing ration size and oyster weight are unreasonably high for oysters in the lower weight category. Optimum daily rations of 1.5 to 4.9 % of algae to body weight per day have been reported for oysters (Enright *et al.*, 1986; Urban and Pruder, 1992). At

an available ration equivalent to 1.5 to 2.0 % of body weight per day rock oysters selected for growth grew approximately four times the rate of the control oysters (Bayne, 2000). The optimal ration might also be affected by culture conditions influencing the efficiency with which the food is utilized, such as concentration and quality of the algae, stocking density and condition (Coutteau, *et al.*, 1994).

Food availability is regarded as one of the most important factors influencing the growth of bivalves. Bayne and Newell (1983) suggest that to attain high growth rate, molluscs may show increased oxygen consumption and feeding to attain high energy turn over by maximizing ingestion, despite the increased faecal and metabolic losses. The present study shows that feeding and absorption in *P. fucata* is significantly correlated with food availability. Adjusting the feeding behavior under different food conditions to meet physiological requirements is common in many bivalve species (Hawkins, *et al.*, 1997; Cranford *et al.*, 1998; Wong and Cheung, 1999). Also, the feeding variables such as clearance rate, absorption rate and absorption efficiency are related to the total particulate matter present in sea water.

Pseudofaeces production is a kind of selection behavior in bivalves. Although it is generally believed that pseudofaeces are produced in bivalves at high levels of total particulate matter, this selective behavior has also been observed at low concentrations (Pouvreau, *et al.*, 2000). In the present study, almost no pseudofaeces was produced at 15 cells/μl, 30 cells/μl and 60 cells/μl concentrations of food. Its production was, however, noticed when algal concentrations are at higher levels. High concentration appears to result in elevated filtration rates in *P. fucata* and as a result pseudofaeces production. Although the mechanisms involved in preferential ingestion are not fully understood, gill structure, innervation, muscular control of the gill filaments, particle size and structure, chemosensory capabilities, gut residence time, digestive enzymes and mucous secretions may all be important as mentioned by Shumway, *et al.* (1990).

The relative food value of the algal species examined were in the order *I. galbana* > *C. calcitrans* > *S. costatum* > *N. salina* > *T. gracilis*. Walne (1970) has found very high growth rates of the clam *Mercenaria mercenaria* fed with *S. costatum* while Epifanio (1979) showed that *T. suecica* promoted moderate growth of *M. mercenaria* juveniles. One of the more important factors determining nutritional value of alga is their polyunsaturated fatty acid (PUFA) content (Langdon and Waldock, 1981; Webb and Chu, 1983). In particular eicosapentaenoic acid, 20:5 ω3 and docosahexaenoic acid, 22:6 ω3 are considered to be essential. Algae, which contain relatively high amounts of one or the other generally, support much better growth than species without these fatty acids. *S. costatum* and *C. calcitrans* which are rich in 20:5 ω_3 are of good food value. The good growth of pearl oyster spat on feeding with *Chaetoceros calcitrans* in the present study may be due to the presence of eicosapentaenoic acid 20:5ω_3 and docosahexaenoic acid 22:6ω_3 in the microalgae. These algae can also be cultured successfully on a large scale, both in intensive indoor systems and in outdoor tanks, making them particularly useful for commercial pearl oyster rearing. Algae, *S.costatum, N. salina and T.gracilis,* which indicated moderate to low growth rate, may lack essential PUFA's and may have low cell wall digestibility. Although, variation in ambient water temperatures was kept low during the study, increase in temperature also affects food consumption, absorption efficiency and respiration (Laing *et al.*, 1987). Laing *et al.* (1987) have also reported that some algae such as *C. calcitrans* are utilized more efficiently for growth by juveniles of *Ostrea edulis*. The results of the present study also show that certain algal diets promote good growth in *P. fucata* spat, with *I. galbana* and *C. calcitrans* being especially suitable either singly or in combination. For commercial onshore culture of the pearl oyster, a balance needs to be maintained between growth rate, growth efficiency and the species of algae used.

5. 3. BIOCHEMICAL COMPOSITION OF MICROALGAE AND SPAT

The biochemical composition of algae varies significantly among species. Fernandez-Reiriz *et al.* (1989) have pointed out that it is not easy to compare data on the biochemical composition of phytoplanktonic algae. Differences in the culture conditions, in the analytical methods or in the growth phase sampled make it difficult to compare the results presented by different authors. For example, they quote protein values ranging from 3.19 to 67.10% dry weight for *I. galbana* and 2.60 to 48.61 dry weight for *C. calcitrans*. In *Isochrysis galbana* Fabregas *et al.* (1986) has found a low protein content of 3.19 to 5.34% organic weight, while Ben-Amotz *et al.* (1985, 1987) have recorded a high levels of 23.3 to 37.9% organic weight and Fernandez-Reiriz *et al.* (1989) have observed the protein contents to vary over a wide range of 13.29% to 39.97%. Utting (1985) has noted still higher values of 41.7% to 67.1%. Antia *et al.* (1963) and Myklestad and Laing (1972) have stated the large variation in the protein levels may be due to exhaustion of nutrients in the culture medium or accumulation of metabolites. In the present work the protein levels of the five species of microalgae have been observed to vary over a limited range of values of 23-29% dry weight and near to those, 23-30 reported by Ben-Amotz *et al.* (1985).

The lipid content of *I. galbana* (17%) dry weight observed in the present study is lower than most values reported in previous works in the species (Utting, 1985; Whyte, 1987; Fernandez-Reiriz *et al.*, 1989). Utting (1985) has found the lipid level of *I. galbana* to range from 20.10 to 31% organic weight, Whyte (1987) has reported 24-25.55% and Fernandez-Reiriz *et al.* (1989) 25.88% to 36.16%. The lipid level of *Chaetoceros calcitrans* noted in the present work, 19% is much higher than 9.89% to 11.76% quoted by Fernandez-Reiriz *et al.* (1989) but lower than values of 20.70% reported by Ben-Amotz *et al.* (1987) and 21-57 to 26.95% recorded by Whyte (1987). Lipid content has been found to be lowest, 8-6% in *Skeletonema costatum* and *Tetraselmis gracilis* respectively in the present work.

The carbohydrate content of microalgae studied in the present work is much lower than the levels observed in the same species by previous workers. This may be due to difference in climatic conditions. While the carbohydrate level of *I. galbana* in the present study is 4% dry weight that in the same species has been found to be 22.80% to 34.50% by Utting (1985), 11 to 20.50% by Ben-Amotz *et al.* (1985, 1987), 20.10% by Fabregas *et al.* (1986) and 15.21% to 48.35% by Fernandez-Reiriz *et al.* (1989). In the present work the carbohydrate level of *Chaetoceros calcitrans* is 5%. Compared to this, the carbohydrate content of the same species has been noted by Fernandez-Reiriz *et al.* (1989) to be 2.42% at exponential phase at 20°C, 11.32% in early stationary phase and 8.36% in late stationary phase.

The protein/carbohydrate ratio in the microalgae has been found to range in the present work from 3.13 for *N. salina* to 5.75 in *I. galbana*. These values are higher than those reported for the same species and other species of algae (Myklestad, 1974; Hitchcock, 1980; Fabregas *et al.*, 1985a,b; Fabregas *et al.*, 1986). Low values are obtained when there is depletion of nitrogen in the culture medium. Variability in the biochemical composition of *I. galbana* and other microalgae has been shown to result from environmental factors. Variations are also closely associated with changes in nutrient concentration, which in turn reflect in the protein and carbohydrate values. Fabregas *et al.* (1986) have observed that the variability in the protein content per cell is probably the most important factor in aquaculture systems. They caution that any mariculture system using microalgae as feed could be affected by these variations.

Carbohydrate content of *T. gracilis* was higher than that of other species with the exception of *N. salina*. But the lipid content of *T. gracilis* was the lowest among the species studied. Otero and Fabregas (1997) have found that carbohydrates and not lipid were accumulated as energy storage under nitrogen stress in the semicontinuous cultures of *Tetraselmis suecica*. The chief storage product of the algal cells was lipid in each species except *T. gracilis*, which contained slightly more carbohydrate than fat. Parsons *et al.* (1961) have found carbohydrate to be the storage product except in the case

of *Chaetoceros* sp. Sukenik and Wahnon (1991) have stated that *I. galbana* accumulates carbohydrates under high-light or nitrogen-limiting conditions suggesting that *I. galbana* stores polysaccharide as an energy storage material, while lipids are mainly used as structural components of cellular membranes.

The gross composition of algae studied is in the range reported for 40 species by Brown *et al.* (1997) who have found that diatoms contain more lipid than other algae. This is true in the present case with both *C. calcitrans* and *S. costatum*. Algal diets rich in polyunsaturated fatty acids and with high levels of carbohydrate have been reported to produce the best growth for juvenile oysters, scallops and mussels (Enright *et al.*, 1986b; Whyte *et al.*, 1989; Kreeger and Langdon, 1993). Volkman *et al.* (1989) have suggested that nutritional deficiencies in a diet can be avoided by the use of mixed algal diets.

Gross chemical composition (protein, carbohydrate, lipid) of different algae significantly influences the growth of juvenile molluscs. Piveteau *et al.* (1999) have found that supplying *Skeletonema costatum* to oysters largely improved the condition index of the oysters. Wikfors *et al.* (1984) have suggested that the increased efficiency of one food source over another indicate a probable causal relationship between algal lipid content and oyster growth. The biochemical composition of food source may also be altered on assimilation by the oysters. Lewin *et al.* (1979) have concluded that fatty acid composition of clam being different from that of its food sources is indicative of an active fatty acid metabolism that changes both chain length and degree of saturation. Knauer *et al.* (1999) have found that after a six-week feeding period, the sterol profile of spat tissues generally reflect that of the diet. In the present study, *P. fucata* spat fed with microalgae differing in biochemical profiles were healthy and grew well with differences in relation to algal species.

SUMMARY

1. 'Nursery rearing of pearl oyster *Pinctada fucata* (Gould) under onshore conditions' comprises a critical evaluation of the nutritional value of microalgal diets for spat growth. Several sets of experiments each with specific objective have been carried out in the shellfish hatchery at Visahkapatnam Regional Research Centre of Central Marine Fisheries Research Institute.

2. In the present study, the optimum spat density observed was 20/60l. A significant aspect of the results in the present study is the 100% survival rate observed even in higher densities like 80/60l and 160/60l.

3. At densities of 40/60 l, 80/60 l and 160/60 l, growth of spat decreased with increasing density. A statistically precise correlation could be calculated between DVM and individual weight of spat with DVM as a good index of growth.

4. Except for *I. galbana* and *S. costatum*, the concentration of 60 cell/µl resulted in maximum growth of spat in single species diets. Low food concentrations of about 15 and 30 cells/µl, resulted low growth.

5. The decreasing growth rates observed in the present study with increase algal concentration above 60 cells /µl suggests that the pearl oyster is not adapted for efficient feeding at high food concentrations above 60/µl. This may probably be due to reduction in filtering efficiency and/or pumping rate and increase in production of pseudofaeces.

6. In the present study, spat were fed twice daily with a gap of 8 hours between feeding. Filtration efficiency is efficient with discontinuous feeding, possibly because the microalgae supplied after an interval stimulates filtration, and this, in turn, results in net gain to the spat in terms of consumption.

7. The two species diet of *C. calcitrans* and *I. galbana* showed a better growth rate than when fed as individual diets. The same also holds good for other combinations of three species diets or a combination of all the five algal species, which showed remarkable growth.

8. It is not only the presence or absence of certain essential components in the diet, but in attaining the correct balance between them, which will promote growth. The different growth rates observed in *P. fucata* fed on mixed algal diets, points to a possibility of selective removal of particular components, and ingestion of materials that are quantitatively more important.

9. The relative food value of the algal species examined were in the order *I. galbana*>*C. calcitrans*>*S. costatum*>*N. salina*>*T. gracilis*.

10. In the present work the protein levels of the five species of microalgae have been observed to vary over a limited range of values of 23-29% dry weight. The lipid levels of *C. calcitrans, I. galbana, N. salina, S. costatum* and *T. gracilis* are in the descending order 19%, 17%, 12%, 8% and 6%respectively. The carbohydrate levels of *C. calcitrans, I. galbana, N. salina, S. costatum* and *T. gracilis* in the present study are lower 5%, 4%, 8%, 6% and 7% dry weight respectively.

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